40472

National Library of Canada / Bibliothèque nationale du Canada

CANADIAN THESES ON MICROFICHE / THÈSES CANADIENNES SUR MICROFICHE

NAME OF AUTHOR/*NOM DE L'AUTEUR* OUELLET MARIE

TITLE OF THESIS/*TITRE DE LA THÈSE* THE MEASUREMENT OF THE EMULSIFYING CAPACITY OF MEAT PROTEINS

UNIVERSITY/*UNIVERSITÉ* ALBERTA

DEGREE FOR WHICH THESIS WAS PRESENTED/ *GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* MASTERS

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE GRADE* 1979

NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* DR. F.H. WOLFE

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

DATED/*DATÉ* April 10 SIGNED/*SIGNÉ* Marie Ouellet

PERMANENT ADDRESS/*RÉSIDENCE FIXÉ* 2960 rue Valmont
Ste-Foy, Québec
G1W 1Z3

NL-91 (3-74)

National Library of Canada

Cataloguing Branch
Canadian Theses Division

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada

Direction du catalogage
Division des thèses canadiennes

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

THE UNIVERSITY OF ALBERTA

THE MEASUREMENT OF THE EMULSIFYING CAPACITY OF MEAT PROTEINS

by

© MARIE OUELLET

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN FOOD SCIENCE

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA

SPRING, 1979

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The Measurement of the Emulsifying Capacity of Meat Proteins

submitted by Marie Ouellet

in partial fulfilment of the requirements for the degree of Master of Science in Food Science

Supervisor

External Examiner

Date Feb 2, 1979

ABSTRACT

The cardiac muscle has been reported to be a poor emulsifier. The present experiments were undertaken in an attempt to evaluate and improve its emulsifying characteristics. The emulsifying capacity (EC), the maximum amount of fat that can be emulsified by a given amount of protein, was measured by an increase in the electrical resistance of an emulsion formed during mixing. Preliminary experiments investigating this technique cast doubt on its reliability.

The EC of buttermilk powder and Bovine Serum Albumin (BSA) solutions, skeletal and cardiac muscle slurries, sarcoplasmic and myofibrillar protein solutions was measured under various conditions of concentration, pH, ionic strength, oil addition and initial volume using three mixers. No difference was found in EC between skeletal and cardiac muscles measured in a Waring Blendor. No reproducible results could be obtained with the Lightnin stirrer because of lack of control over the speed of mixing. Using a Stedi Speed stirrer, the EC of cardiac muscle was slightly higher than that of skeletal muscle and sarcoplasmic proteins were more efficient than myofibrillar proteins.

The conclusion is that a new method should be developed to evaluate the emulsifying properties of proteins to be used in emulsion products.

ACKNOWLEDGMENTS

I wish to thank the National Research Council of Canada for financial support in the form of a 1967 Science Scholarship.

I am also grateful to Dr. F. H. Wolfe, my supervisor, also for financial assistance, and for his helpful suggestions in the preparation of the manuscript.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
Emulsifying Capacity in Meat Emulsion Formulation	1
Nature of Meat Emulsions	2
Model Systems	3
Use of model systems	3
Basic principles	5
Most currently used systems	6
New model systems	7
Factors Affecting the Emulsifying Capacity	9
Effect of time of comminution	9
Effect of protein concentration	9
Effect of initial volume (solution and oil)	16
Effect of speed of mixing	17
Effect of rate of fat addition	19
Effect of temperature	20
Effect of different types of fats	22
Effect of method of detection of the end-point	22
Effect of units	24

Page

Emulsifying Characteristics of Meat Protein Fractions 24

Microscopic examination of protein membranes 26

Effect of salt 26

Effect of pH 28

Effect of protein concentration 29

Salt-soluble proteins as emulsifying agents 29

Emulsifying Characteristics of Poultry Meat 31

Water- and salt-soluble proteins 31

Light meat and dark meat 33

Effect of pH 34

Various classes of poultry 35

Prerigor and postrigor meat 36

Type of tissue 37

Others factors 37

Emulsifying Characteristics of Other Protein Sources 38

Protein additives 38

Fish proteins 40

Meat by-products 41

Classification of Meats as Binders 42

Classification according to sausage makers 42

Classification based on EC measurements 43

Comparison of skeletal and cardiac muscles 43

Statement of the Problem 43

Page

MATERIALS AND METHODS 46

Preparation of Meat Slurries 46

Method I 46

Method II 47

Method III 47

Preparation of Protein Solutions 48

Extraction of Meat Proteins 48

Sarcoplasmic proteins 48

Myofibrillar proteins 48

Method I 48

Methods II and III 49

Connective tissue proteins 49

pH Adjustment 49

Protein Determination 49

Proteolysis 50

Preblending 50

Viscosity 50

Preparation of a Tripolyphosphate Solution 51

Measurement of EC 51

Method I 51

Method II 52

Method III 54

Page

RESULTS AND DISCUSSION 57

Method I 57

Preliminary experiments 57

Comparison of different kinds of proteins 57

Effect of enzymatic digestion 59

Effect on a skeletal muscle slurry 59

Comparison between skeletal and cardiac muscle slurries 60

Effect of protein concentration on EC 60

Buttermilk powder 60

Skeletal and cardiac muscle slurries 62

Sarcoplasmic and myofibrillar proteins 74

Effect of preblending 74

Effect of pH 76

Problems associated with Method I 77

Method II 78

Preliminary experiments 78

Effect of salt 79

Effect of buffer 80

Effect of rate of oil addition 83

Effect of pH 83

Effect of speed of mixing 86

Effect of initial volume of solution 88

Effect of protein concentration 89

Problems associated with Method II 94

Page

Method III 95

Effect of rate of oil addition 96

Effect of initial volume of solution 96

Effect of initial volume of oil 97

Effect of salt 101

Effect of pH 103

Effect of tripolyphosphate 103

Effect of protein concentration 106

BSA solutions 107

Skeletal and cardiac muscle slurries 107

Sarcoplasmic and myofibrillar proteins 115

Effect of preblending 119

Effect of enzymatic digestion 126

Evaluation of Method III 130

SUMMARY AND CONCLUSIONS 132

BIBLIOGRAPHY 142

LIST OF TABLES

Table	Description	Page
1	Effect of time of comminution of meat slurry on EC	10
2	Effect of protein concentration on EC	10
3	Effect of initial amount of oil in the mixer	17
4	Effect of rate of mixing on EC	18
5	EC of water- and salt-soluble proteins	32
6	Effect of pH of broiler breast muscle on EC	34
7	Effect of pH on EC of light (L), dark (D) and skin (S) tissues of different classes of poultry	35
8	Effect of prerigor, postrigor and stored broiler meat on EC	36
9	Effect of age and pre- and postrigor turkey breast muscle on EC	36
10	Effect of skin content prior to deboning on EC of mechanically deboned meat	38
11	EC of various sausage meats	44
12	EC of different kinds of protein by Method I	58
13	Effect of enzymatic digestion on EC of skeletal muscle by Method I	59
14	Effect of enzymatic digestion on EC of skeletal muscle by Method I	61
15	Effect of enzymatic digestion on EC of cardiac muscle by Method I	61
16	Effect of protein concentration on EC by Method I (buttermilk)	62

Table	Description	Page
17	Effect of protein concentration on EC by Method I (skeletal muscle)	64
18	Effect of protein concentration on EC by Method I (cardiac muscle)	64
19	Effect of protein concentration on EC by Method I (skeletal muscle)	69
20	Effect of protein concentration on EC by Method I (cardiac muscle)	69
21	EC of skeletal muscle protein fractions by Method I	75
22	EC of cardiac muscle protein fractions by Method I	75
23	Effect of pH on the EC of skeletal and cardiac muscle slurries by Method I	76
24	Effect of NaCl concentration on the amount of oil added to a salt solution by Method II	79
25	Effect of KCl concentration on the amount of oil added to a salt solution by Method II	80
26	Effect of buffer concentration on the amount of oil added to a buffer solution by Method II	81
27	Effect of rate of oil addition on the amount of oil added to a 1,0 M NaCl solution by Method II	84
28	Effect of pH on the amount of oil added to a 1,0 M NaCl solution by Method II	86
29	Effect of speed of mixing on the EC of a myofibril solution by Method II	87
30	Effect of initial volume of solution on the EC of a myofibril solution and a cardiac muscle slurry by Method II	88
31	Effect of protein concentration on EC of a myofibril solution by Method II	92
32	Effect of rate of oil addition on the amount of oil emulsified by a 0,02% BSA solution by Method III	97

Table	Description	Page
33	Effect of initial volume of solution on the amount on oil emulsified by a 0,02% BSA solution by Method III	99
34	Effect of initial volume of oil on the amount of oil emulsified by a 0,02% BSA solution by Method III	101
35	Effect of salt concentration on the amount of oil added to salt solutions with and without protein by Method III	102
36	Effect of pH on the amount of oil added to salt solutions with and without protein by Method III	104
37	Effect of tripolyphosphate concenération on the amount of oil added by Method III	106
38	Effect of protein concentration on EC by Method III (BSA)	107
39	Effect of protein concentration on EC of skeletal muscle slurry by Method III	109
40	Effect of protein concentration on EC of cardiac muscle slurry by Method III	110
41	Effect of protein concentration on EC of skeletal muscle slurries by Method III	116
42	Effect of protein concentration on EC of cardiac muscle slurries by Method III	116
43	Effect of protein concentration on EC of sarcoplasmic proteins from skeletal muscle by Method III	120
44	Effect of protein concentration on EC of sarcoplasmic proteins from cardiac muscle by Method III	120
45	Effect of protein concentration on EC of myofibrillar proteins from skeletal muscle by Method III	123
46	Effect of protein concentration on EC of myofibrillar proteins from cardiac muscle by Method III	123

Table	Description	Page
47	Effect of enzymatic digestion on viscosity and EC of a skeletal muscle slurry by Method III	127
48	Effect of enzymatic digestion on viscosity and EC of a cardiac muscle slurry by Method III	127
49	Factors producing a large variation in EC as measured by Method I	132
50	Factors producing little or no variation in EC as measured by Method I	133
51	Effect of various factors on EC as measured by Method II	134
52	Effect of various factors on EC as measureb by Method III	135
53	Characteristics of myosin emulsions prepared with various oil-water ratios	140

LIST OF FIGURES

Figure		Page
1	Effect of protein concentration on EC of myosin and sarcoplasmic proteins	12
2	Effect of variable amounts of soluble protein in a meat slurry on the amount of oil emulsified	13
3	Effect of protein concentration on EC and emulsified volume	14
4	Effect of protein concentration on EC of animal protein isolates	15
5	Effect of initial volume of soluble protein extract on the amount of oil emulsified	16
6	Effect of speed of mixing on the amount of oil emulsified by a protein extract	18
7	Effect of rate of fat addition on the amount of fat emulsified	20
8	Effect of maximum temperature of the emulsion on the amount of fat emulsified	21
9	Effect of pH on the percent oil phase volume of emulsion at inversion	25
10	Effect of pH and NaCl concentration on the amount of fat emulsified by water-soluble proteins	27
11	Effect of pH and NaCl concentration on the amount of fat emulsified by salt-soluble proteins	28
12	Effect of protein concentration on EC for poultry meat extracts	33
13	Effect of protein concentration on EC and the amount of oil emulsified for a buttermilk solution by Method I	63

Figure		Page
14	Effect of protein concentration on EC of skeletal and cardiac muscle slurries before preblending by Method I	65
15	Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries before preblending by Method I	66
16	Effect of protein concentration on EC of skeletal and cardiac muscle slurries after preblending by Method I	67
17	Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries after preblending by Method I	68
18	Effect of protein concentration on the EC of skeletal and cardiac muscle slurries before preblending by Method I	70
19	Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries before preblending by Method I	71
20	Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries after preblending by Method I	72
21	Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries after preblending by Method I	73
22	Effect of buffer concentration on the amount of oil added to a KCl-tris buffer by Method II	82
23	Effect of rate of oil addition on the amount of oil added to a 1,0 M NaCl solution by Method II	85
24	Effect of initial volume of solution on EC and the amount of oil emulsified for a myofibril solution by Method II	90
25	Effect of initial volume of solution on EC and the amount of oil emulsified for a cardiac muscle slurry by Method II	91
26	Effect of protein concentration on EC and the amount of oil emulsified for a myofibril solution by Method II	93

Figure		Page
27	Effect of rate of oil addition on the amount of oil emulsified by a 0,02% BSA solution using by Method III	98
28	Effect of initial volume of solution on the amount of oil emulsified by a 0,02% BSA solution by Method III	100
29	Effect of pH on the amount of oil added to salt solutions with and without protein by Method III	105
30	Effect of protein concentration on EC and the amount of oil emulsified for BSA solution by Method III	108
31	Effect of protein concentration on EC of skeletal and cardiac muscle slurries before preblending by Method III	111
32	Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries before preblending by Method III	112
33	Effect of protein concentration on EC of skeletal and cardiac muscle slurries after preblending by Method III	113
34	Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries after preblending by Method III	114
35	Effect of protein concentration on EC of skeletal and cardiac muscle slurries before preblending by Method III	117
36	Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries before preblending by Method III	118
37	Effect of protein concentration on EC of sarcoplasmic proteins by Method III	121
38	Effect of protein concentration on the amount of oil emulsified by sarcoplasmic proteins by Method III	122
39	Effect of protein concentration on EC of myofibrillar proteins by Method III	124
40	Effect of protein concentration on the amount of oil emulsified by myofibrillar proteins by Method III	125

Figure		Page
41	Effect of proteolysis on the viscosity of skeletal and cardiac muscle slurries	128
42	Effect of proteolysis on the EC of skeletal and cardiac muscle slurries by Method III	129
43	Comparison between the EC of sarcoplasmic proteins from skeletal and cardiac muscles as determined by the Waring Blendor (Method I) and the propeller (Method III) techniques	137
44	Comparison between the EC of myofibrillar proteins from skeletal and cardiac muscles as determined by the Waring Blendor (Method I) and the propeller (Method III) techniques	138

LIST OF PLATES

Plate		Page
1	Experimental set up (Method I)	53
2	Experimental set up (Method II)	55
3	Experimental set up (Method III)	56

INTRODUCTION

Emulsifying Capacity in Meat Emulsion Formulation

The production of a meat emulsion involves the chopping of lean meat with salt, other seasonings and ice to extract the salt-soluble proteins. Fat is then added and chopped into small globules that are surrounded by a protein membrane; for lack of more definitive terminology these mixtures are called emulsions. It has long been recognized that the meats used in these mixtures differ in their capacity to stabilize emulsions, that is to bind the fat, and this variability represents one of the major problems of meat emulsion production.

In the past decade, a great deal of effort has been expended in the attempt to better understand the emulsification process; much information was obtained using model systems. The effects of processing variables have been determined, meats have been ranked according to their ability to stabilize emulsions and numerical binding values have been assigned to them which are used in linear programming of meat emulsion formulations. With the introduction of new protein sources, it was thought to be useful to be able to quantitate their emulsifying properties as compared to meat proteins. The most currently used model system measures the maximum amount of fat that can be emulsified by a given amount of a particular protein. This quantity is known as emulsification capacity.

Nature of Meat Emulsions

> *An emulsion is a heterogenous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, whose diameters, in general, exceed 0,1 μ. Such systems possess a minimal stability, which may be accentuated by such additives as surface-active agents, finally-divided solids, etc..*

This is the definition that Becher (1965) gives of an emulsion.

There has been controversy over the past several years as to whether or not sausage meat batters should be considered as meat emulsions. Because of its complexity such a system is, strictly speaking, not a true emulsion. However, since fat exists as dispersed small droplets enveloped by a layer of protein, the system constitutes essentially an oil-in-water emulsion and the term meat emulsion is generally accepted. This was established in 1960 by Hansen who first published photomicrographs of experimental meat mixtures; he showed that the salt-soluble proteins formed an appreciable membrane around the fat globules. Other workers using microscopy found that highly comminuted meat products were similar to classical oil-in-water emulsions consisting of fat droplets surrounded by a membrane of protein material (Carpenter and Saffle 1964; Helmer and Saffle 1963; Meyer *et al.* 1964; Swift *et al.* 1961). Electron microscopy revealed that even very small fat globules (0,1 μ in diameter) had distinct protein membranes (Borchert *et al.* 1967).

Model Systems

Use of model systems

Until two decades ago, manufacture of meat emulsion products was mainly an empirical science. The various factors affecting the formation and stability of meat emulsions were studied under practical conditions using production-type equipment. The disadvantages in using such an approach are numerous. For example, it is very difficult to carry out reliable full-scale experiments because of such factors as variation in raw materials and difficulty in controlling factors other than the variable being tested in such a complex system as a meat emulsion. Therefore, the phenomena observed are very difficult to interpret and the information obtained from such tests is of limited application. Furthermore, such experiments are time-consuming.

As a result of the above mentioned limitations, model systems had to be used to investigate the role of the various ingredients. One of the major problems in manufacture of meat emulsion products is the retention of fat. Three different measures were developed to determine the ability of the proteins to emulsify fat namely, emulsifying capacity (EC), emulsion stability (ES) and emulsifying ability (EA). In this review, we are mainly concerned with the EC which is the most commonly used method. The effect of various factors such as the type of meat, protein and fat, pH, shear force, temperature and others were determined by various workers by measuring the EC.

But model systems are not without problems. Since the environment is different in a complete food system, results obtained from a model system cannot always be related to practical conditions. So as pointed out by Mattil (1971), the question must always be asked:

> *What is the effect of the other components of the system ?*
> *How do the data from an emulsifiability test run in a beaker relate to the complete ionic environment in a frankfurter ?*

In the system of Swift *et al.* (1961), emulsion collapse occurred at a protein to fat ratio of 1:1600, whereas in actual practice a ratio of 1:4 will never be exceeded. In model systems, the emulsions are exposed to a stress (speed and time of mixing) far beyond the one produced by commercial equipment. Another problem is the variety of methods used by various researchers producing data which are difficult to compare. Even the terminology used in the literature is confusing, EC having a different meaning for different workers and being expressed in different units.

This emphasizes the need for improved standardized quantitative laboratory methods to evaluate the properties of conventional and new proteins and determine the effect of different treatments on the functionality of these proteins. So many variables are involved in the actual production of a meat emulsion that it is almost impossible to develop a laboratory method with a congruent relationship to the actual practice. But it should be possible to obtain results which can be correlated to practical situations (Inklaar and Fortuin 1969). The most currently used procedure is the method of Swift *et al.* (1961).

Basic principles

As has already been mentioned, manufacture of meat emulsion products was empirical not long ago. Need for increased understanding and better quality stimulated research in the area of emulsion science. Out of this interest a number of methods were described to measure the emulsification properties of proteins. The first model system was developed by Swift *et al.* in 1961 and the basic principles involved are still the same in the systems now in use.

The system devised by Swift *et al.* (1961) measured the so-called emulsifying capacity. This method determined the maximum amount of fat that could be emulsified by a protein under standard conditions. It was based on the fact that the breakdown of a meat emulsion usually results in separation of fat during processing.

The basic method described by Swift *et al.* (1961) consisted of blending at high speed a fixed amount of meat slurry or protein extract of known concentration together with a fixed initial aliquot of melted lard. A thick oil-in-water emulsion resulted. As mixing was continued, more lard was incorporated into the emulsion at a specific rate until the emulsion inverted i.e. until the viscosity dropped sharply. The amount of lard expressed in ml per unit of protein was the emulsifying capacity (EC) of that protein.

This method with modifications has been widely used as an indicator of the relative value of proteins as emulsifiers. Several modifications based on this model system have been reported either as minor changes or important modifications. Most modifications resulted from the use of

different equipment and consisted of changes in speed of mixing, rate of oil addition, means of detection of the end-point, protein concentration and others. The resulting emulsions were visually different and fat globule sizes were also different (Saffle 1968). The fact that EC has been measured in different ways by different authors makes the comparison between studies difficult. Since EC depends on the system used each system should be described thoroughly.

Most currently used systems

In the first model system developed by Swift *et al.* in 1961 a muscle protein slurry was prepared by mixing 50 g of ground meat together with 200 ml of cold 1,0 M NaCl solution in a Sorvall Omni-mixer for 2 minutes at 13 000 rpm. The type of container (1 pint jar, diameter 7,3 cm) and the propeller used (blade span 5,7 cm) were described and details concerning the temperature were given, the jar contents being immersed in an ice bath. Twelve and five-tenths grams of slurry were diluted with 37,5 ml of cold saline solution and mixed for a few seconds at 1 000 rpm. A specific amount of melted lard (50 ml) was added to the diluted slurry and high speed mixing (13 000 rpm) was started. A stiff oil-in-water emulsion was formed and became more and more viscous on continued fat addition at a rate of 0,8 ml per second. The breaking of the emulsion was indicated by a sudden drop in viscosity, at which point the addition of fat was stopped. The emulsifying capacity was defined as the total amount of fat added in ml of fat per 2,5 g of tissue.

Carpenter and Saffle (1964) attempted to develop a standard procedure for laboratory determination of the comparative emulsifying capacity of various meat proteins. Although they referred to their method as being different it was actually only a modification of that of Swift *et al.* (1961). They used an Osterizer mixer, and an inverted pint Ball jar to which 25 ml of protein extract (10 mg/ml) and 50 ml of Wesson oil were added and blended for 30 seconds at 13 400 rpm. More oil was then added at a constant rate of 0,50 ml per second until the viscosity suddenly dropped. Carpenter and Saffle (1964) had no problem with the types of rigid emulsions resistant to mixing reported by Swift *et al.* (1961). The main difference between the two systems resides in the fact that Carpenter and Saffle (1964) worked with salt-soluble protein extracts instead of meat slurries.

As mentioned earlier, there exists a different method for each group of workers. Therefore, the following discussion will be based on results obtained by different researchers referring to their own system. Unless otherwise stated, the basic principles involved are the same as in the model of Swift *et al.* (1961).

New model systems

In a recent study using chicken breast muscle myosin, Galluzzo and Regenstein (1978a) optimized the conditions for the EC test with respect to temperature of oil, rate of mixing, oil addition rate and aqueous starting volume. Based on these conditions, they developed a timed emulsification test in which fixed amounts of aqueous and oil phases were emulsified for fixed periods of time. The changes occurring in the

various proteins during emulsification were followed by SDS gel electrophoresis. Using this system they investigated the role of the various meat proteins in the emulsification process for purified proteins (Galluzzo and Regenstein 1978b) and more complex model systems, namely, natural actomyosin and glycerinated contracted and uncontracted myofibrils (Galluzzo and Regenstein 1978c). Of the purified proteins, myosin was the most rapidly taken up into the emulsion followed by actomyosin and then actin. In more complex systems they found that myosin was again preferentially used over actin. According to their relative loss of solubility at the oil/water interface they ranked the myofibrillar proteins, actin being the most resistant followed by tropomyosin and troponin with actomyosin and myosin coming next and being roughly equal.

Schut (1978) developed a new model system which created a link between model systems where oil was emulsified and actual meat emulsion production. In this system, rendered solid pork fat was emulsified in a matrix consisting of a special type of pre-gelatinized starch (for sufficient resistance to achieve a comparable reduction of animal fat during chopping) in various meat protein extracts at low temperature. This method reproduced more closely the situation in a bowl chopper and although more research is necessary in this particular area this is the kind of system that is desirable. The remainder of this review deals with the basic system first developed by Swift *et al.* (1961).

Factors Affecting the Emulsifying Capacity

The factors that can affect the EC of meat proteins were studied by several researchers, in particular by Swift *et al.* (1961) and Carpenter and Saffle (1964). Conditions such as equipment design, shape of container, speed of blending, rate of oil addition, temperature, pH, protein (source, solubility and concentration), kind of oil used, salt (type and concentration), sugar and water content all affect the EC of proteins (Saffle 1968).

The following factors affecting EC measurement were studied: time of comminution of the meat in saline solution, dilution of the meat slurry, initial volume of slurry and oil, emulsification at different rates of mixing and different rates of oil addition, temperature and effectiveness of water- and salt-soluble proteins. The EC of various sausage meats was also determined.

Effect of time of comminution

Swift *et al.* (1961) found that there was an optimum time of comminution of the meat in saline solution of two minutes for which the EC was maximum (table 1, from Swift *et al.* 1961).

Effect of protein concentration

It was also found by Swift *et al.* (1961) that as the protein concentration decreased, the EC increased and the viscosity of the emulsion formed decreased. Their results are presented in table 2.

TABLE 1. Effect of time of comminution of meat slurry on EC

Time of comminution at 13 000 rpm	EC
min	ml oil/2,5 g tissue
1	137
2	148
3	133
4	131

(From Swift *et al.* 1961, table 1)

TABLE 2. Effect of protein concentration on EC

NaCl/2,5 g tissue	EC	Description of the emulsion
ml	ml oil/2,5 g tissue	----
22,5	82	semi-solid
47,5	127	viscous, mixable
100,0	179	moderately viscous

(From Swift *et al.* 1961, table 2)

This effect of protein concentration was observed by other workers using both model systems for meat (Gillett *et al.* 1977; Hegarty *et al.* 1963; Ivey *et al.* 1970; Maurer *et al.* 1969a; Tsai *et al.* 1972; Young 1976) and non meat proteins (Pearson *et al.* 1965; Wang and Kinsella 1976) and also for laboratory prepared meat emulsions (Morrison *et al.* 1971). Kinsella (1976) attributed the effect of protein concentration to a greater degree of unfolding of the protein chains caused by the shearing

force and hydrophobic bonds with lipid droplets resulting in a greater ratio of volume to surface area and hence a greater emulsification capacity.

Whereas the negative relationship between EC and protein concentration was curvilinear in most cases (figure 1, from Tsai *et al.* 1972), Carpenter and Saffle (1964) contended that a straight-line relationship existed as long as the system was not overloaded (figure 2, from Carpenter and Saffle 1964).

This gave rise to some controversy in the literature (Gillett *et al.* 1977). However, it can be shown that the confusion arose from the way of expressing the results. Acton and Saffle (1972) found that the positive linear relationship obtained by Carpenter and Saffle (1964) was also negative and curvilinear when the results were expressed as amount of oil per unit of protein (figure 3, from Acton and Saffle 1972). Because part of the emulsion remained on the side of the jar and because the mixing was incomplete near the end-point at high protein concentration, Saffle (1968) attributed the decrease in EC to overloading of the system. Subsequently, Ivey *et al.* (1970) suggested that thicker protein membranes were formed on the fat globules at high protein concentrations utilizing more protein per drop. As it was explained by Gillett *et al.* (1977), the EC and the amount of oil emulsified are only different algebraic expressions of the same data, this fact is illustrated in figure 3 in which the line for the volume of oil becomes the axis of the parabola formed by the EC curve. In his work on animal protein isolates, Young (1976) failed to find a curvilinear relationship (figure 4, from Young 1976) because even the more dilute isolates did not fall upon the oil volume curve.

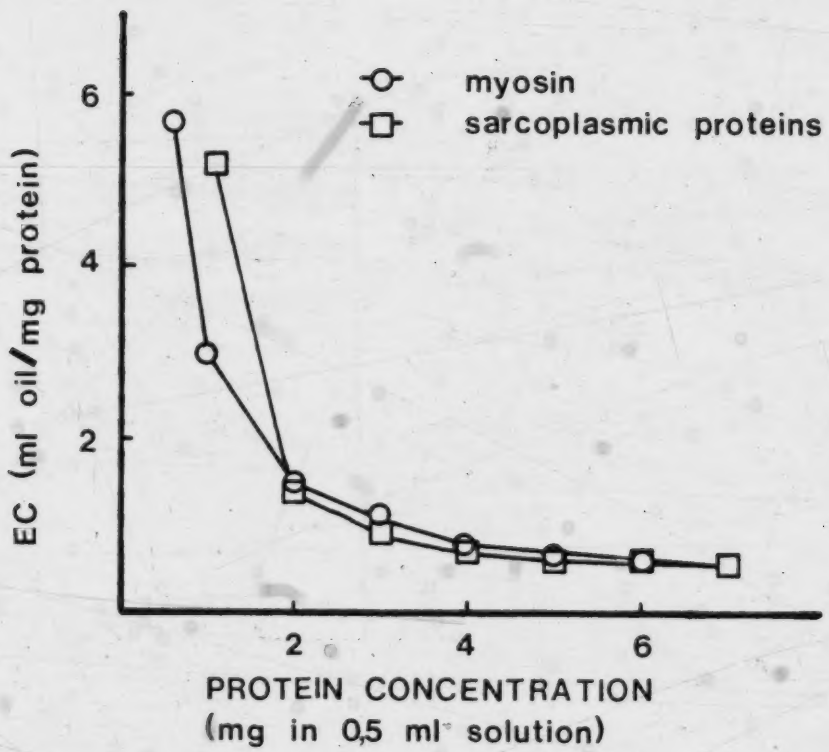

Figure 1: Effect of protein concentration on EC of myosin and sarcoplasmic proteins. Salt concentration of solutions is 0,5M KCl (from Tsai *et al.* 1972, figure 2)

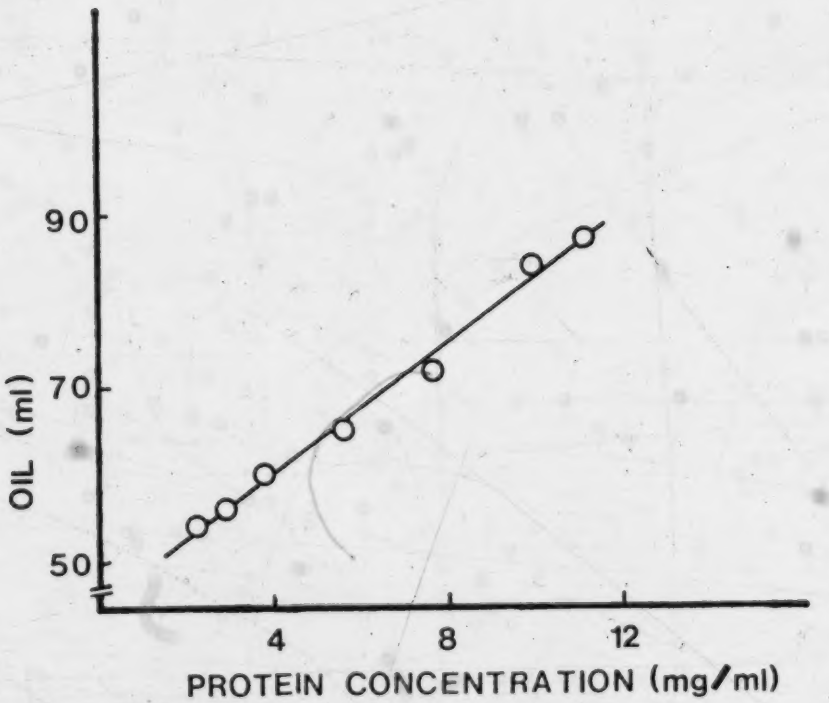

Figure 2: Effect of variable amounts of soluble protein in 25 ml portion of meat slurry on the amount of oil emulsified (from Carpenter and Saffle 1964, figure 3)

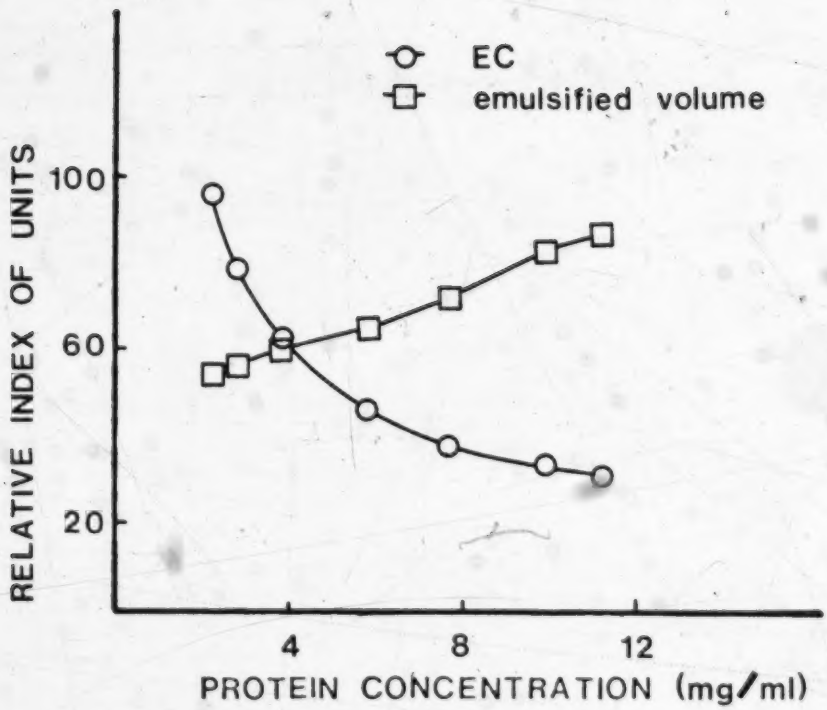

Figure 3: Effect of protein concentration on EC (ml oil/100 mg protein) and emulsified volume (total ml of oil) (from Acton and Saffle 1972, figure 2)

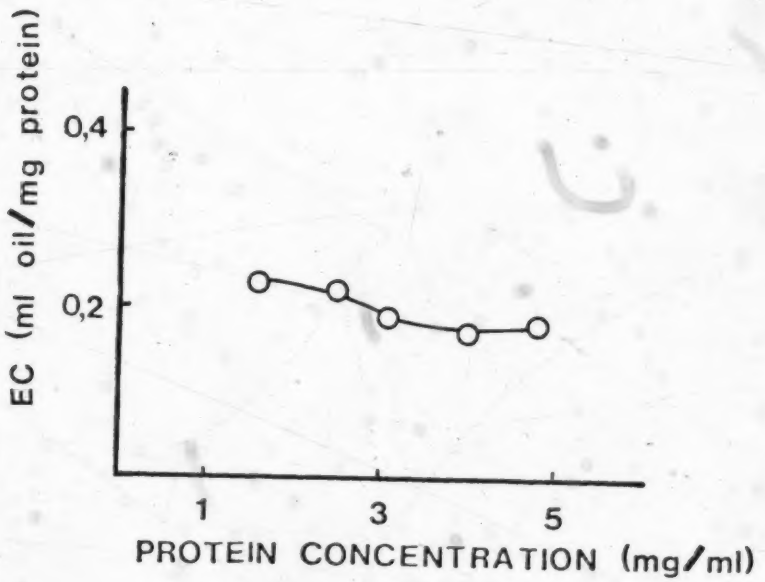

Figure 4: Effect of protein concentration on EC of animal protein isolates (from Young 1976, figure 2)

Effect of initial volume (solution and oil)

Carpenter and Saffle (1964) found that the EC increased linearly with the initial amount of soluble protein extract (figure 5, from Carpenter and Saffle 1964). As the initial volume of oil was increased the EC also increased. The effect of the initial amount of oil was in fact attributed to differences in final temperature resulting from longer times of exposure to the shearing force (table 3, from Carpenter and Saffle 1964).

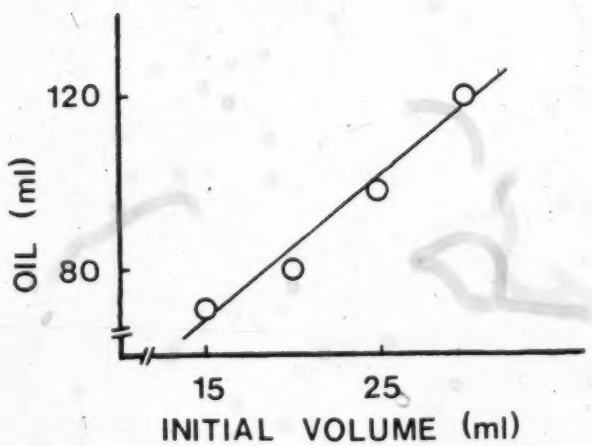

Figure 5: Effect of initial volume of soluble protein extract (containing 11,3 mg of soluble protein per ml) on the amount of oil emulsified (from Carpenter and Saffle 1964, figure 1)

TABLE 3. Effect of initial amount of oil in the mixer

Initial amount of oil	EC	Final temperature
ml	ml oil/100 mg soluble protein	°C
25	30,79	31-33
50	33,20	27-28
60	33,20	27-28
75	no emulsion formed	---

(From Carpenter and Saffle 1964, table 1)

Effect of speed of mixing

Several workers reported that the speed of mixing inversely affected the amount of oil that could be emulsified by meat proteins (Carpenter and Saffle 1964; Crenwelge *et al.* 1974; Ivey *et al.* 1970; Swift *et al.* 1961). Moreover, Swift *et al.* (1961) observed that the rate of mixing also affected the character of the emulsion produced, its viscosity increasing with the rate of mixing (table 4, from Swift *et al.* 1961). Carpenter and Saffle (1964) found an inverse linear relation between EC and the speed of mixing provided that the temperature at emulsion collapse was controlled (figure 6, from Carpenter and Saffle 1964); the oil was dispersed into smaller droplets leading to an increasing surface area of the oil to be emulsified by the same limiting quantity of protein. The effect of the speed of mixing was also confirmed by Hegarty *et al.* (1963) who worked at much lower speed and found higher values of EC. Using the system of Hegarty *et al.* (1963),

TABLE 4. Effect of rate of mixing on EC

Rate of mixing	EC	Description of the emulsion
rpm	ml fat/2,5 g tissue	----
13 000	142	viscous, mixable
9 200	168	slightly viscous
6 500	185	grainy suspension

(From Swift *et al.* 1961, table 2)

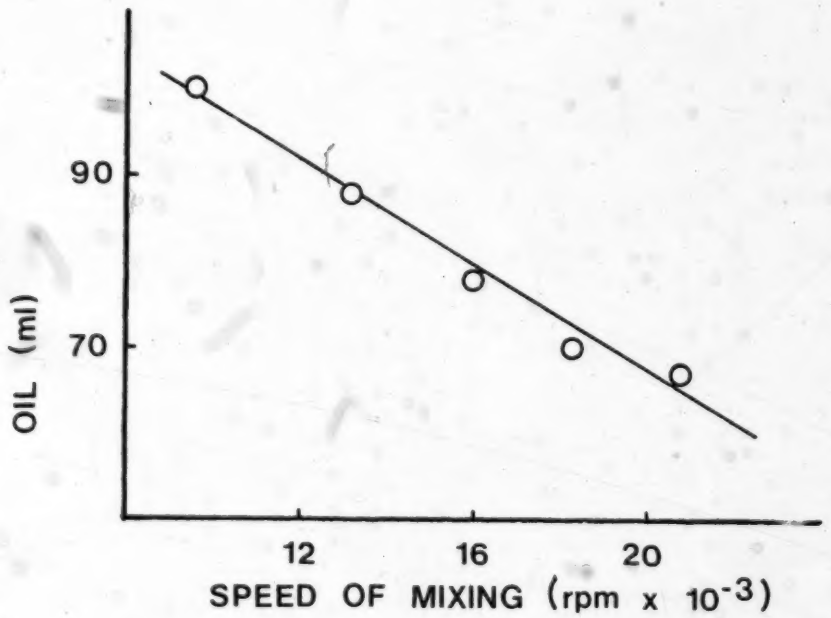

Figure 6: Effect of speed of mixing on the amount of oil emulsified by a protein extract containing 11,3 mg soluble protein/ml (from Carpenter and Saffle 1964, figure 2)

Pearson *et al.* (1965) clearly showed that the fat globules were considerably larger than those obtained from other systems with higher rates of mixing.

Ivey *et al.* (1970) discussed the effect of the speed of mixing in terms of the inter-relationships between droplet size and interfacial film thickness. Schut (1976) also provided an explanation for the effect of shear force: high speed mixing for a prolonged period of time could impair the mechanical properties of the films explaining the inverse relation between EC and speed of mixing. Crenwelge *et al.* (1974) reported that maximum emulsification occurred at different speeds depending on the protein source. According to these researchers optimum blender speeds should be used; however, under these conditions, exact comparison with other published results would be impossible. That time and type of mixing had an important effect on the character of the emulsion was further demonstrated by Tornberg and Hermansson (1977) who compared the emulsions produced by different types of mixers.

Effect of rate of fat addition

The effect of rate of fat addition is not clear. Swift *et al.* (1961) reported a positive linear relationship between rate of fat addition and amount of oil emulsified (figure 7, from Swift *et al.* 1961). Huffman *et al.* (1975) demonstrated an optimum rate which was different for each protein source. On the other hand, Carpenter and Saffle (1964) reported no effect of the rate of oil addition unless the capacity of the mixer was exceeded or unless the different rates resulted in different temperatures at emulsion collapse.

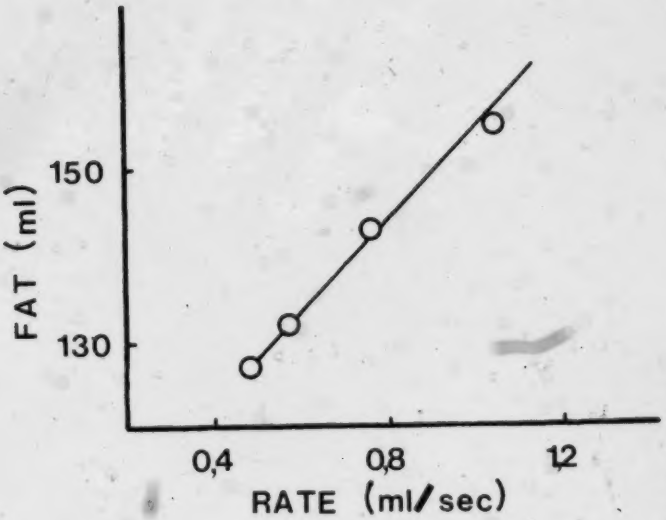

Figure 7: Effect of rate of fat addition on the amount of fat emulsified (from Swift *et al.* 1961, figure 2)

Effect of temperature

The practical experience of sausage makers who take many precautions to prevent excessive temperature rise during chopping was substantiated by the results obtained in model systems. Swift *et al.* (1961) observed an inverse linear relationship between the temperature reached during emulsification and the EC of meat proteins within a range of 18 to 46°C (figure 8, from Swift *et al.* 1961). Carpenter and Saffle (1964) found a similar negative relation in a range from 4 to 50°C. They stated that the final temperature of the emulsion was more critical than factors like the rate of oil addition or the speed of mixing; uncontrolled temperature would change the otherwise straight-line relationship between EC and protein concentration to a curvilinear

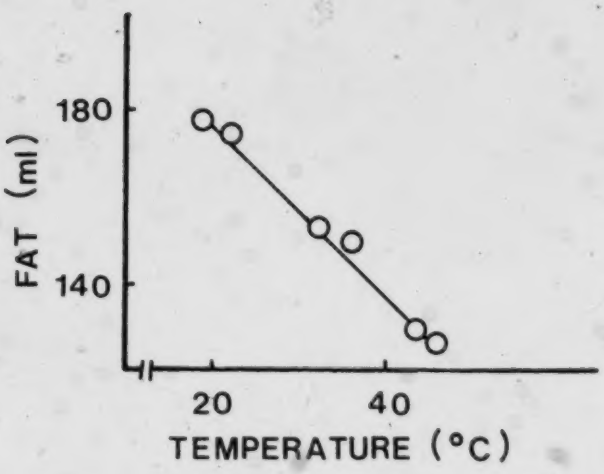

Figure 8: Effect of maximum temperature of the emulsion on the amount of fat emulsified (from Swift *et al.* 1961, figure 3)

one as reported for other systems. At that time the influence of temperature was explained by an increase in interfacial area, a decrease in viscosity, the expansion of oil drops, denaturation of the proteins, and promoted coalescence of oil drops at increasing emulsification temperature (Schut 1976). Other studies showed that heat denaturation could not account for the effect of temperature. For example, the EC of a salt-soluble protein extract held at 38°C for more than 3 hours was unaffected by this treatment (Schut 1976). Using meat emulsions, reversibility of the emulsifying properties was found after chilling of a previously heated emulsion (Helmer and Saffle 1963). Schut (1976) did not find a linear relationship between temperature and EC but found it was true that the size of the fat globules was reduced at higher temperatures

resulting in an increased surface area. According to this researcher, the effect of temperature could be explained in terms of film elasticity and viscosity and emulsion viscosity which all decreased at high temperature impairing emulsion stability. More information on the effect of temperature was obtained with actual meat emulsions (Helmer and Saffle 1963; Saffle *et al.* 1967; Townsend *et al.* 1968) but it will not be detailed here.

Effect of different types of fats

The behaviour of different plant and animal fats and oils in emulsions produced in a model system was studied by Christian and Saffle (1967). Among the saturated fatty acids and triglycerides the lower molecular weight species could be emulsified in larger amounts. At comparable fatty acid chain length, more unsaturated than saturated fatty acids were emulsified with fatty acids with one double bond coming first followed by those with two double bonds. Significant differences were found in the amounts of the different plant oils and animal fats which could be emulsified by meat protein extracts. However, the results obtained from this study were not applicable to actual production for two reasons. First, only a small increase in the fat level could be obtained by selecting the type of fat and secondly, the fats which could be added in larger amounts were different from those which would have been predicted from results of experiments with pure fatty acids.

Effect of method of detection of the end-point

The determination of the end-point is another factor that can affect the EC. Several researchers used the sudden drop in viscosity

which occurred at emulsion collapse as the indicator of the end-point (Borton *et al.* 1968; Carpenter and Saffle 1964, 1965; Christian and Saffle 1967; Ivey *et al.* 1970; Pearson *et al.* 1965; Swift *et al.* 1961; Swift and Sulzbacher 1963). This method was reported to be subjective and not useful for emulsions of low viscosity and to require an experienced operator (Webb *et al.* 1970; Marshall *et al.* 1975). A number of other methods were suggested for the determination of the end-point. Webb *et al.* (1970) measured the resistance of meat emulsions, which remained low until the fat droplets began to aggregate where it increased very sharply. However, emulsions of low viscosity were believed to distort the determination because of their electrophoretic properties and emulsions of high viscosity had a tendency to coat the electrodes and to increase the resistance (Haq *et al.* 1973). Haq *et al.* (1973) used direct current resistance measurements to determine emulsion stability but noted that a few problems were associated with the method which could be solved in part by the use of alternating current.. Others (Crenwelge *et al.* 1974; Tybor *et al.* 1973) used a sudden drop in amperage required to drive the mixer motor as an indicator of emulsion oil titration end-point. However, this method was dependent on the viscosity changes occurring at emulsion collapse which made it of little use for emulsions of either low or high viscosity (Marshall *et al.* 1975). Recently, Marshall *et al.* (1975) developed a technique where the visual identification was facilitated by the increased visibility of coloured oil droplets.

Effect of units

There are no standardized conditions for the determination of EC, the various investigators establishing conditions arbitrarily. Furthermore, EC has been usually expressed in different units. As a result, comparisons between studies are virtually impossible. Acton and Saffle (1972) proposed the use of the oil phase volume at emulsion collapse defined as the percent oil volume of the total emulsion volume to compare data from different studies. According to these workers, the constancy of the oil phase volume of muscle proteins would minimize the effect of protein concentration (figure 3, from Acton and Saffle 1972). However, the oil phase volume was later found to be variable by other researchers (Crenwelge *et al.* 1974; Tybor *et al.* 1973). Tybor *et al.* (1973) reported that proteins from beef blood varied in oil phase volume at different concentrations; in certain cases, they varied from about 40 to 85% oil phase volume. Proteins from cottonseed and soybean seeds, non fat dry milk and globin were found to vary in oil phase volume over a wide range of pH values (figure 9, from Crenwelge *et al.* 1974).

Emulsifying Characteristics of Meat Protein Fractions

The first mention of the different ability of the various meat proteins to emulsify fat was made in 1959 by Turner and Olson who obtained a patent based on the fact that myofibrillar proteins regulate the stability of meat emulsions. Since then, the importance of the myofibrillar proteins as emulsifying agents has been reported by several researchers. A few of these studies will be reported here which emphasize the fact that water-soluble protein films are

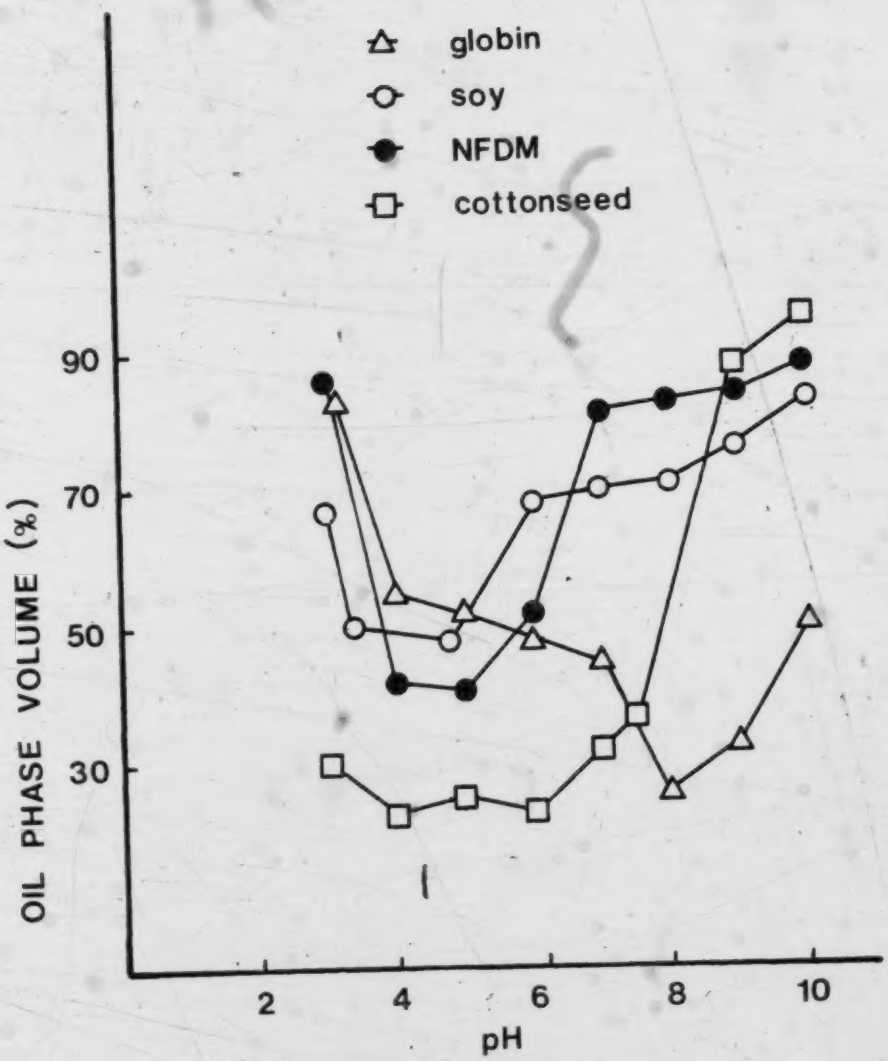

Figure 9: Effect of pH on the percent oil phase volume of emulsion at inversion (from Crenwelge *et al.* 1974, figure 3)
NFDM: non fat dry milk

characteristic of interfacial protein films whereas salt-soluble proteins exhibit a behaviour different from that of any other protein. The optimum condition for formation of protein films is high ionic strength at the isoelectric point (Schut 1976).

Microscopic examination of protein membranes

As has already been mentioned, microscopic examination of emulsions indicated that salt-soluble proteins formed thicker membranes around the fat globules than water-soluble proteins (Hansen 1960; Swift *et al.* 1961; Trautman 1964).

Effect of salt

Swift *et al.* (1961) reported that both water- and salt-soluble proteins are capable of stabilizing emulsions but to a different extent. Even though they found the ability of water-soluble proteins to stabilize emulsions to be enhanced by salt, it always remained inferior to that of salt-soluble proteins. In a subsequent study, Swift and Sulzbacher (1963) further demonstrated the enhancing effect of salt on the EC of the water-soluble proteins (figure 10, from Swift and Sulzbacher 1963). The effect of salt on water-soluble proteins was explained in terms of critical surface area which also increases with increasing ionic strength of the water phase, corresponding to a greater extent of spreading of the protein molecule (Schut 1976). Swift and Sulzbacher (1963) also showed that anions affected EC in the order of the Hofmeister series (KSCN, KI, KNO_3, KBr, KCl and K_2SO_4 in decreasing order), probably according to their ability to influence the unfolding of the protein

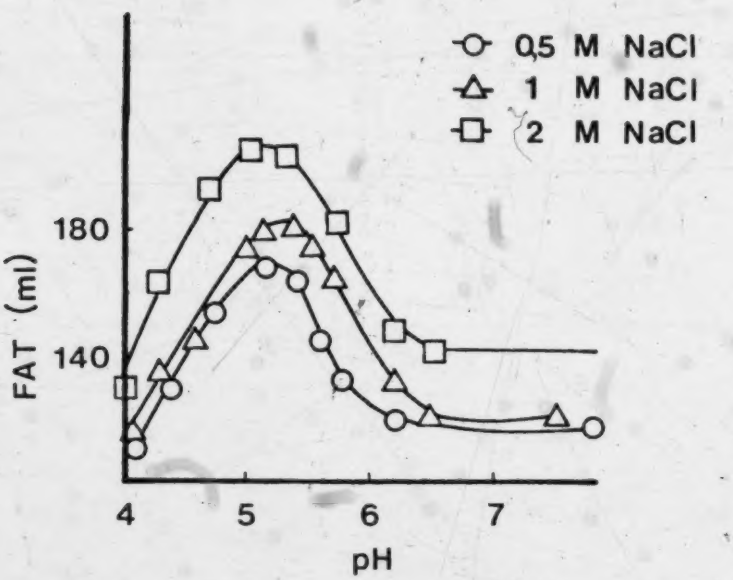

Figure 10: Effect of pH and NaCl concentration on the amount of fat emulsified by water-soluble proteins (from Swift and Sulzbacher 1963, figure 1)

molecules. The importance of the configuration of the protein molecule on EC was further demonstrated by Du Bois *et al.* (1972) who experimented with enzymatic hydrolysis of meat proteins. Limited proteolysis increased EC but prolonged proteolysis caused a decrease. On the other hand, the EC of salt-soluble proteins was found to increase with increasing ionic strength only in the pH range between 5.0 and 6.0 (figure 11, from Swift and Sulzbacher 1963). Swift and Sulzbacher (1963) attributed this effect of ionic strength on EC to changes in solubility of the proteins.

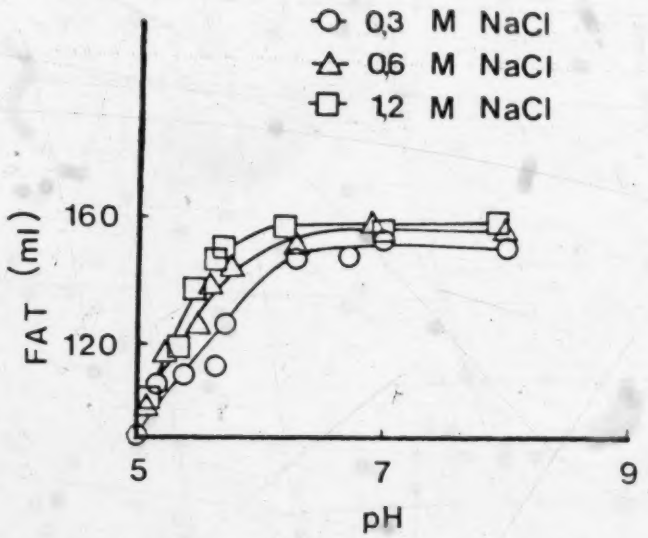

Figure 11: Effect of pH and concentration of NaCl on the amount of fat emulsified by salt-soluble proteins (from Swift and Sulzbacher 1963, figure 2)

Effect of pH

Swift and Sulzbacher (1963) found the highest EC of water-soluble proteins at pH 5.2, which corresponded to their isoelectric point; their EC decreased on either side of this pH value (figure 10, from Swift and Sulzbacher 1963). The maximum value of EC has been attributed to the formation of salt bridges and hydrogen bonds which is also maximum at the isoelectric point (Schut 1976). On the contrary, the EC of salt-soluble proteins reached a maximum value at pH 6.0 and 6.5 and remained constant as the pH was increased to 8.0 in the study of Swift and Sulzbacher (figure 11, from Swift and Sulzbacher 1963).

Effect of protein concentration

Swift *et al.* (1961) found that the EC of the salt-soluble protein increased on dilution whereas the efficiency of the water-soluble proteins remained approximately the same. Schut (1978) observed that the protein load, defined as mg of protein absorbed per gram of oil, decreased as the protein-to-oil ratio was increased for the salt-soluble proteins but remained constant for the water-soluble proteins. On the other hand, it was also reported that EC also decreased with increasing protein concentration for water-soluble proteins (figure 1, from Tsai *et al.* 1972).

Salt-soluble proteins as emulsifying agents

Evidence of the importance of the salt-soluble proteins as emulsifying agents has been provided by several researchers. Fukazawa *et al.* (1961), working with experimental sausages, reported an appreciable decrease in binding quality when myosin was removed. However, sausages made from actin-poor fibrils or without water-soluble proteins were only slightly inferior to those prepared from the whole muscle.

Hegarty *et al.* (1963) ranked the different intracellular beef muscle proteins from greatest EC to least as follows: actin in the absence of salt, myosin, actomyosin, sarcoplasmic proteins and actin in 0,3 M salt. In other words, myofibrillar proteins had a greater EC than sarcoplasmic proteins. A similar state of affairs was reported for mutton (Baliga and Madaiah 1970) and for porcine muscle (Tsai *et al.* 1972).

In 1964, Carpenter and Saffle developed a method similar to that of Swift *et al.* (1961) based on salt-soluble proteins to determine the EC of sausage meats. While Swift (1965) reported the EC of the salt-soluble proteins to be from 30 to 400% that of water-soluble proteins, Carpenter and Saffle (1964) found the water-soluble proteins were only 70% as efficient in EC as the salt-soluble proteins. The ability of meat to emulsify depended in part on the shape and charge of the protein molecule as shown by Carpenter and Saffle (1965). The superiority of the salt-soluble proteins might be explained in terms of the shape of the molecule since water-soluble proteins are approximately spherical in shape whereas salt-soluble proteins are rather asymetrical molecules.

Using the rate of fat separation as an indicator of emulsifying ability, Trautman (1964) found the salt-soluble proteins to be the major emulsifying components in porcine muscle while the emulsifying power of the water-soluble proteins and of the salt-insoluble residue was very low.

Saffle and Galbreath (1964) used the salt-soluble protein content as a measure of EC. They related the extractability of the salt-soluble proteins from various meats to their known effect in meat emulsions. In general, meat sources producing superior emulsions had higher amounts of extractable proteins.

The postmortem decrease in emulsifying efficiency for bovine (Saffle and Galbreath 1964), porcine (Trautman 1964) and ovine (Van Eerd 1972) muscles was believed to be caused by decreased extractability of the salt-soluble proteins.

A high correlation (r = 0,94) was found between the amount of soluble protein from different meat sources and the emulsifying properties of their extracts by Gillett *et al.* (1977). Freezing and cooking altered both the solubility of the proteins and their emulsifying capacity.

In a very recent study comparing results obtained from practical experiments and those obtained from two model systems, Schut (1978) concluded that the salt-soluble fraction plays the important role in emulsion formation rather than the water-soluble protein fraction, confirming results from previous reports by other researchers. Schut ranked the meat proteins in decreasing order of EC as follows: myosin, actomyosin and water-soluble proteins. He also reported preferential absorption of the proteins at the interface in the same order. While the water-soluble protein fraction showed no preferentially absorbed protein, the salt-soluble protein fraction consisted of several sub-fractions with different values of EC.

Emulsifying Characteristics of Poultry Meat

Water- and salt-soluble proteins

So far, we have been mostly concerned with beef meat. Much work has also been done using poultry meat. At low protein concentration, the EC of water- and salt-soluble proteins was the same; however, the salt-soluble proteins were more efficient than the water-soluble proteins at high protein concentrations (table 5, from Maurer *et al.* 1969a). The same workers could not find any difference in EC when it was measured

TABLE 5. EC of water- and salt-soluble proteins

Protein concentration	Water-soluble proteins	Salt-soluble proteins
mg/ml	ml oil/100 mg protein	
2	43,5	48,0
5	19,6	24,4
10	10,8	18,1
15	7,7	15,2

(From Maurer *et al.* 1969a, table 1)

using protein solutions of equal concentration from different parts of the carcass. It was also reported that the EC of both water- and salt-soluble proteins was higher at low protein concentration (figure 12, from Maurer *et al.* 1969a). Maurer *et al.* (1969a), Parkes and May (1968), and Neelakantan and Froning (1971) evaluated the EC of some intracellular turkey muscle proteins at pH 6.0 and 7.0. The EC of myosin and actomyosin was highest at both pH levels. At pH 7.0 the EC of the sarcoplasmic proteins was lowest but at pH 6.0 their EC was similar to that of actomyosin.

In an histological investigation of poultry meat emulsions, Froning *et al.* (1970) found that the removal of the salt-soluble proteins greatly changed the characteristics of the emulsion whereas extraction of the water-soluble proteins did not visibly affect the prepared emulsion as judged by a paraffin embedding technique. The salt-soluble proteins were apparently the principal emulsifying agent at the surface of the fat globule. The removal of the salt-soluble proteins resulted in large fat globules and protein inclusions within them; fat globules

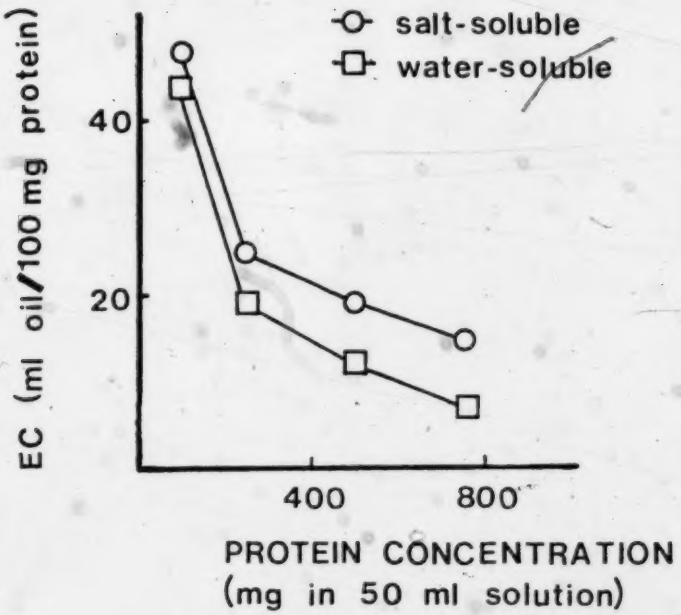

Figure 12: Effect of protein concentration on EC for poultry meat extracts (from Maurer *et al.* 1969a, figure 1)

were also more irregular. It was believed that the amount of salt-soluble proteins was insufficient to cover the fat globule surface thereby allowing coalescence of the fat globules. Their data emphasize the role of the salt-soluble proteins in meat emulsions.

Light meat and dark meat

Although the total protein and salt-soluble protein levels were higher in light meat, the EC of dark meat was equal or higher than that of light meat for all classes of poultry (Hudspeth and May 1967; McCready and Cunningham 1971b; Parkes and May 1968). The greater amount of available soluble protein in light meat was counter-balanced

by the greater EC of soluble protein from dark tissue. As pointed out by McCready and Cunningham (1971b), the superiority of dark meat might be due to the significantly lower pH of light meat.

Effect of pH

The influence of pH on EC of poultry was studied by several researchers who all found a higher EC at higher pH. The EC of broiler breast muscle was higher at pH values above pH 5.9 (table 6, from Froning and Neelakantan 1971). Using meats from various classes of poultry, McCready and Cunningham (1971b) reported that EC measured at pH 7.0 was significantly greater than EC at normal pH or pH 5.0 (table 7, from McCready and Cunningham 1971b); as mentioned earlier, the greater EC of dark meat over light meat could be related to the higher pH of the former. The same thing was true of broiler meat (McCready and Cunningham 1971a).

TABLE 6. Effect of pH of broiler breast muscle on EC

	EC ml fat/2,5 g tissue			
	Fresh		Frozen storage 6 months at -29°C	
pH	< 5.9	> 5.9	< 5.9	> 5.9
prerigor	156	171	---	---
postrigor	134	151	142	155

(From Froning and Neelakantan 1971, table 2)

TABLE 7. Effect of pH on EC of light (L), dark (D) and skin (S) tissues of different classes of poultry

pH	EC ml oil/2 g tissue											
	turkey toms			turkey hens			broilers			fowl		
	L	D	S	L	D	S	L	D	S	L	D	S
normal	108	118	51	139	140	54	143	169	73	130	137	40
5.0	84	76	41	83	64	46	92	73	57	80	62	34
7.0	150	149	51	190	185	63	204	210	73	66	187	45

(From McCready and Cunningham 1971b, table 3)

Various classes of poultry

EC was found to be different for various classes of poultry. The value of EC of meat from turkey hens was about equal to that for fowl; it was higher than that for turkey toms and slightly lower than that of broilers (table 7, from McCready and Cunningham 1971b). That meat from broilers had a superior EC when compared to that from fowl and turkeys was true both on a protein basis (Hudspeth and May 1967; Maurer *et al.* 1969a) and a weight basis (McCready and Cunningham 1971b). The comparison of turkey and fowl is not as easy; on a protein basis, turkey meat was reported to have similar (Hudspeth and May 1967) or better EC (Maurer *et al.* 1969b).

Prerigor and postrigor meat

Meat in the prerigor state had an EC superior to that of postrigor muscle as demonstrated using breast muscle from broilers and turkeys (tables 6, 8 and 9 from Froning and Neelakantan 1971).

TABLE 8. Effect of prerigor, postrigor and stored broiler meat on EC

	EC ml oil/2,5 g breast meat
prerigor	167
postrigor	145
6 months storage	145

(From Froning and Neelakantan 1971, table 1)

TABLE 9. Effect of age and pre- and postrigor turkey breast muscle on EC

Age weeks	State of rigor	EC ml oil/2,5 g meat
22	pre	145
	post	133
24	pre	161
	post	130
26	pre	185
	post	137

(From Froning and Neelakantan 1971, table 3)

Type of tissue

The type of tissue also had an effect on the EC. The EC of skeletal tissue from various classes of poultry was found to be higher than that of other tissues containing larger amounts of connective tissue (table 7, from McCready and Cunningham 1971b; Hudspeth and May 1969; Maurer and Baker 1966).

Other factors

The EC of poultry meat was also affected by several other factors. Addition of salt improved EC (Maurer *et al.* 1969a). The EC of prerigor turkey meat increased with age from 22 to 26 weeks (table 9, from Froning and Neelakantan 1971). Freeze drying and rehydration of salt-soluble protein extracts from broiler meat did not significantly affect their EC (Parkes and May 1968). Frozen storage of broiler meat did not significantly lower the EC (tables 6, 8, from Neelakantan and Froning 1971). The EC of mechanically deboned poultry meat (MDPM) was reported to be the same as that of hand deboned meat (Noble 1973). A number of factors could affect the EC of MDPM. The EC of MDPM was significantly decreased on a meat basis as the skin content was increased (table 10, from Froning *et al.* 1973). This was found to be due to an excess of fat (Satterlee *et al.* 1971) and then confirmed with MDPM from fowl meat whose EC was greater after centrifugation to remove excess fat and heme pigments (Froning and Johnson 1973).

TABLE 10. Effect of skin content prior to deboning on EC of mechanically deboned meat

Skin content %	EC	
	ml fat/2,5 g meat	ml fat/mg protein
0	182	0,50
16,8	148	0,48
28,9	133	0,47
38,9	127	0,50

(From Froning *et al.* 1973, table 2)

Emulsifying Characteristics of Other Protein Sources

Because of the projected shortage of meat proteins, a special emphasis has been placed on the properties of new sources of proteins, particularly on their functional properties. Numerous researchers evaluated the emulsifying properties of new protein sources using a variety of EC techniques. A few investigations will be reported here.

Protein additives

Protein additives are of particular interest as substitutes for meat. Pearson *et al.* (1965) measured the EC of the following protein additives: soy sodium proteinate, potassium caseinate and non fat dry milk (NFDM). The solubility of these proteins was closely related to the values of EC. The pH was also found to influence the EC, probably in an indirect manner by affecting the solubility of the proteins.

Because the results obtained from EC determinations were not applicable to practical conditions, Inklaar and Fortuin (1969) developed a different model system. Their system, involving cooking and centrifugation, gave results similar to those found in actual practice. They reported the various factors affecting the emulsion stability of protein meat additives. The parameters involved included dispersion time, amount of oil, protein and water, temperature, speed of mixing and pH of the system. They reported the factors affecting the solubility of the myofibrillar proteins and related the emulsification test to the amount of soluble proteins. Using the same method, Lin *et al.* (1975) studied the quality of wieners supplemented with sunflower and soy protein products.

Another type of model system was developed for the study of the emulsifying properties (emulsifying activity and emulsion stability) of soy protein concentrates (Yasumatsu *et al.* 1972). A negative correlation was found with the crude protein content and a positive correlation with soluble nitrogen. This confirmed the conclusions of Johnson and Henrickson (1970) and Inklaar and Fortuin (1969) who also found that nitrogen solubility was a good indicator of the emulsifying properties of soy proteins; Smith *et al.* (1973) found the same thing using a wide variety of non meat protein additives.

Huffman *et al.* (1975) measured the EC of sunflower proteins using 6% suspensions at pH values ranging from 5.7 to 10.8. Different rates of mixing as well as different rates of oil addition were investigated. Optimum EC was observed at pH 7 with speed 4 500 rpm. High mixing speeds lowered the EC.

The emulsifying properties of a number of other protein additives were determined. The effect of various sources and levels of cottage cheese whey in bologna product was determined using EC and other tests together with taste panel evaluation of the finished product; spray-dried commercial whey could be added to the meat mixture at a level of 3,5%. Quinn and Beuchat (1975) utilized the method of Carpenter and Saffle (1964) to study the changes in the functional properties resulting from fermentation of peanut flour; in general, fermentation increased EC and nitrogen solubility of the peanut flour. Conditions of protein concentration, pH, salt concentration, mixing speed and particle size for optimum EC were determined for groundnut protein (Ramanatham *et al.* 1978).

Fish proteins

Fish proteins could also be important to solve the problem of meat protein shortage. Knowledge of their functional properties is essential before fish proteins can be used in food products; their EC was reported by a number of researchers. Grabowska and Sikorski (1974) measured the EC of the codfish proteins and found that the myofibrillar proteins were far superior in emulsifying ability than the other muscle proteins. Frozen storage decreased the EC and this was correlated with a decrease in protein solubility. Minimum emulsification occurred at pH 4.5. The effect of storage conditions on the EC of hydrolyzed fish proteins were studied by Koury and Spinelli (1975) who reported a gradual loss which they attributed to a loss in protein solubility. They showed that several low molecular weight carbohydrates significantly reduced EC. Again with fish protein isolates, changes in organoleptic quality and

loss of solubility coincided with a decrease in EC (Spinelli *et al.* 1972a, 1972b). A limited hydrolysis of the samples up to about 15 minutes increased EC; after that period of time no further increase was obtained.

Meat by-products

And finally, meat by-products should also be taken into consideration. Satterlee *et al.* (1973) measured the EC of high protein powders from beef and pork by-products. They reported that the soluble protein of these powders was responsible for their ability to emulsify fat. According to these researchers, powders containing the greatest amount of soluble protein had the greatest ability to emulsify not only because they contained more protein but also because the proteins were more efficient. The reverse was reported to be true of powders containing small amounts of proteins.

Blood proteins from slaughter animals are another potential source of protein. These proteins were found to be good emulsifiers. Tybor *et al.* (1973) showed that plasma and globin isolates were excellent emulsifiers under optimum conditions of pH and protein concentration. The maximum capacity occurred at a protein concentration of 0,5 g/100 ml. The blood serum proteins exhibited similar values of EC irrespective of drying conditions. The EC of the bovine globin was compared by Crenwelge *et al.* (1974) to that of other proteins under standardized conditions. The three other proteins under study were cottonseed flour, soybean concentrate and non fat dry milk (NFDM). The globin was a good emulsifier when compared to NFDM and the cottonseed

isolate. At low pH values, a relatively high EC was obtained for all proteins but the cottonseed isolate. With increasing pH, the EC decreased to a minimum value but then increased again. The curves obtained were typical of the protein solubility curves; there existed a general correlation between EC and solubility. The optimum protein concentration was dependent on the protein source. According to these researchers, valid comparisons of EC values could be made if the blender speed, protein concentration, rate of oil addition and pH for maximum peptization were optimized conditions for each protein sample.

Those are only a few examples of the extensive use of EC to measure the emulsifying properties of different sources of protein.

Classification of Meats as Binders

Classification according to sausage makers

Sausage making is a way to utilize the trimmings obtained from cutting down the carcass as well as the boneless primal cuts. The latter group contains a greater proportion of myosin, one of the fibrillar protein which has the highest binding properties and therefore helps to stabilize the emulsion. Based on the experience of sausage makers the various sausage meats have been classified into good, medium and poor binders. Trimmings from lean cuts of beef, bull meat and meat from primal cuts such as chuck, plates and flanks are considered as good binders. Under the class of meat with medium binding properties come cheek meat, head meat and lean pork trimmings. Ingredients like hearts, weasand meat, giblets and tongue trimmings

have poor binding qualities whereas fillers like gland meats, brain, lungs, oxlip and tripe have almost no binding quality at all (Baliga and Madaiah 1970).

Classification based on EC measurements

Several researchers have compared the EC of various meat trimmings (Borton *et al.* 1968; Carpenter and Saffle 1964; Gillett *et al.* 1977; Satterlee *et al.* 1973). As mentioned earlier, it is very difficult to compare data from different studies. However, this has been done by Acton and Saffle (1972); the values used by these researchers to which other values have been added are presented in table 11. The difficulty of comparing data is well illustrated in the following table.

Comparison between skeletal and cardiac muscles

The cardiac muscle was not found to be as good an emulsifying agent as the skeletal muscle. These observations made under practical conditions were substantiated by EC measurements. However, while some workers found beef hearts to be very poor emulsifiers, others reported the EC of cardiac muscle to be higher than expected (table 11).

Statement of the Problem

The objective of this research was to elucidate the reasons for the reported lower binding quality of the cardiac muscle as compared to the skeletal muscle in manufacture of meat emulsion products. Since the amount of soluble protein is an important factor in emulsification, are

TABLE II. EC of various sausage meats

Trimmings	EC ml oil/100 mg protein				
	Carpenter and Saffle [1] (1964)	Borton *et al.* [1] (1968)	Acton [1] (1970)	Gillett *et al.* (1977)	Satterlee *et al.* (1973)
cow meat	36,64	---	53,2	---	15,96
pork trimming	36,12	261,4	--	21,76	---
tripe	35,68	---	--	74,33	---
beef cheek	32,72	---	51,6	---	---
beef shank	30,77	---	--	19,88	---
pork cheek	23,40	271,3	48,1	---	---
beef hearts	22,56	273,2	49,6	---	9,57

[1] From Acton and Saffle 1972, table

the stroma proteins contained in the cardiac muscle responsible for its lower quality ? Is there any way to improve the emulsifying properties of the cardiac muscle ? For example, would a controlled enzymatic proteolysis improve its emulsifying characteristics ? These are the main questions for which answers were sought through experimentation. EC was used as the principal investigating tool because, based on the measurement of EC, the various sausage meats had already been assigned binding numbers ranking them for use in linear programming of sausage formulations. However, discrepancies arose when results from the model systems we used were compared to both those from the literature and from actual meat production. Another problem was the lack of reproducibility of the results.

Because of the problems encountered with EC as measured in our laboratory, the following question had to be asked: How should EC be measured in the laboratory ? This is actually the question I have been trying to answer.

MATERIALS AND METHODS

Three methods were used to measure EC. Slight variations in other preparations are mentioned throughout the Materials and Methods section for each EC method.

Preparation of Meat Slurries

The meat was purchased at a local market. Skeletal muscle consisted of boneless bottom round steak which had been shipped to the retailer as vacuum packaged sub primal cuts, and then reduced to retail display size. The age of the samples is unknown but they are not likely more than two weeks old. Cardiac muscle consisted of beef heart which had been previously frozen and then thawed.

Method I

In order to obtain a representative sample, large samples of meat were used. For skeletal muscle, between 100 and 150 g of boneless bottom round steak were used; for cardiac muscle, a whole thawed beef heart went into the preparation of the slurry. In both cases, the meat was minced in a Moulinette food chopper (Moulinex) after which the appropriate volume of 0,6 M NaCl solution was added to attain a muscle to slurry

ratio of 1:5. The skeletal muscle was blended in a Waring Blendor at full speed for 2 minutes, while the cardiac muscle was blended in a King Size Waring Blendor at low speed for the same period of time. Further dilutions were made with 0,6 M NaCl solution. All steps were carried out at 2°C.

Method II

The methods of Swift *et al.* (1961), Borton *et al.* (1968) and Webb *et al.* (1970) were used for the preparation of meat slurries. The method of Borton *et al.* (1968) was finally adopted. It consisted of blending 25 g of ground meat with 100 ml of cold 1,0 M NaCl solution in a Waring Blendor for 2 minutes. To 6,25 g of the slurry were added 37,5 ml of cold 1,0 M NaCl solution. This diluted slurry was used to measure the EC.

Method III

The meat used in the preparation of the meat slurries was treated exactly as if it was to be used for myofibril extraction. The meat was trimmed free of visible fat, chopped into small pieces and minced in the Moulinette food chopper approximately 100 g at a time for three periods of 5 seconds. When necessary, this step was repeated and the two portions were mixed together. Fifty grams of minced meat were weighed out and mixed in a Waring Blendor together with 200 ml of 1,0 M NaCl for three bursts of 15 seconds with 45 seconds between each burst. The slurry was then diluted with more saline solution to a given protein concentration, usually 0,02 g protein/g slurry from which solution other more diluted solutions were prepared. All steps were performed at 2°C.

Preparation of Protein Solutions

Buttermilk solutions were prepared by weighing Northern Alberta Dairy Pool buttermilk powder and adding the appropriate volume of water or NaCl solution to obtain the desired protein concentration. Diluted solutions were prepared from the concentrated stock solution by adding more water or saline solution.

Bovine Serum Albumin (BSA) (fraction V, from Sigma Chemical Company) was used as standard protein. A 2% solution was prepared in 1,0 M NaCl and used as a stock solution; a 0,02% solution was used in most experiments.

Extraction of Meat Proteins

Sarcoplasmic proteins

The sarcoplasmic proteins were extracted according to the procedure of Goll and Robson (1967) as reported by Hay *et al.* (1973) using a tris buffered 0,25 M sucrose extracting solution.

Myofibrillar proteins

Method I

The buffer used to extract the myofibrillar proteins was composed of: 0,6 M KCl, 0,1 M Na pyrophosphate, 0,04 M $NaHCO_3$, 0,01 M Na_2CO_3, at pH 9.2. Proteins were extracted from the pellet resulting from the extraction of the sarcoplasmic proteins with 2½ volumes of the KCl buffer for 1 hour at 2°C under slow paddling conditions. The extract was then centrifuged at 2 500 g for 10 minutes. The supernatant contained the myofibrils.

Methods II and III

Myofibrillar proteins were extracted according to the procedure of Goll and Robson (1967) as reported by Hay *et al.* (1973).

Connective tissue proteins

The extraction procedure is based on the fact that connective tissue proteins are insoluble in an alkaline solution. To a centrifuge bottle were added 20 g of a 1:1 meat slurry prepared with water together with 100 ml of a 0,1 N NaOH solution. The contents were stirred occasionally with a glass rod during incubation at 37°C in a water bath for ½ hour. The mixture was then centrifuged and the supernatant discarded. The incubation and centrifugation steps were repeated twice.

pH Adjustment

The pH of the various protein suspensions, meat slurries and extracts was adjusted approximately to the desired pH with dilute NaOH or HCl solutions using the Fisher Accumet pH meter equipped with a glass electrode. The solutions were kept in the cold room to let them stabilize usually for one or two hours; the pH was then checked and fine adjustment made.

Protein Determination

The amount of protein contained in the various protein suspensions, meat slurries and extracts was determined using the Biuret reagent.

When the protein content of meat slurries was to be determined, a modification of the procedure of Torten and Whitaker (1964) was used. The method had to be modified in order for the slurry to be used instead of the meat itself. Five ml of slurry (1:5) were estimated to be equivalent to 1 g of meat. In order to keep both the concentration and the volume constant, 16 ml of 0,625 ml M NaOH were added to 5 ml of slurry. The rest of the procedure remained unchanged.

In other cases, the protein content was determined according to a standard curve for BSA under the same conditions as the protein under study.

Proteolysis

Papain (Adolph's Meat Tenderizer) was used to study the effect of proteolysis. It was added to pH 6.0 meat slurries at different levels for each experiment so details will be given for each one of them. Unless otherwise stated, the treated samples were kept in the cold room after good mixing of the enzyme powder in the solution. The EC of the treated slurries was determined after different periods of time.

Preblending

Slurries were kept at 2°C for a prolonged period of time to extract the salt-soluble proteins. Unless otherwise stated, the EC of these slurries was measured after 24 hours.

Viscosity

The viscosity of the meat slurries was measured with the slurries at 2°C using a Brookfield Synchro-Lectric viscometer (Brookfield

Engineering Laboratories, Stoughton, Massachusetts) and appropriate spindles.

Preparation of a Tripolyphosphate Solution

A tripolyphosphate solution was prepared by weighing sodium tripolyphosphate and adding the appropriate volume of 1,0 M NaCl solution to obtain the desired concentration. Dilutions were made by adding more saline solution.

Measurement of EC

Three different sets of apparatus were used to measure the EC. They will be referred to as:

Method I: Waring Blendor

Method II: Lightnin stirrer Model F equipped with 3 three-bladed propellers

Method III: Fisher Stedi Speed stirrer.

All three methods are actually modifications of the original system of Swift *et al.* (1961) involving addition of fat to dilute protein suspensions, meat slurries and extracts.

Method I

Two copper electrodes (stranded wire No. 8) were attached to the inside of a Waring Blendor jar. Two holes were drilled through the lid to let the electrodes come out for measurement. Another hole was

made in the lid to allow the addition of Mazola corn oil via a piece of Tygon tubing. The Waring Blendor was connected to a variable auto-transformer (Stagg Inc., Dayton, Ontario). The oil was added using a Buchler Polystaltic pump operated at full speed via a piece of Tygon tubing with the following characteristics (plate 1): I.D. 1/16", O.D. 1/8", wall 1/32". The Waring Blendor was operated at a variable transformer setting of 30 on the 120V scale. Three grams of accurately weighed pH 6.0 slurry usually containing 0,02 g protein/g slurry were added together with 47 ml of 0,6 M NaCl solution to the Waring Blendor jar. The slurry was blended for 30 seconds and then 50 ml of corn oil were added. The contents of the jar were blended for another 30 seconds period after which more oil was added until the emulsion collapsed. The emulsion breakdown was indicated by a change in resistance of the emulsion measured by an Avometer 8 ohmmeter. The entire experiment was performed in the cold room at 2°C and samples, oil and equipment were equilibrated to that temperature before use.

Method II

Two copper electrodes (non stranded wire No. 12) were fitted into a 600 ml beaker. A Lightnin stirrer Model F was used to form the emulsion. At first, the stirrer was equipped with one three-bladed propeller but to solve the mixing problems encountered, three of these propellers had to be stacked on the same shaft; this allowed good mixing when large amounts of Sunberta rapeseed oil were added. The speed of mixing was determined using an Adams Photoelectric tachometer and the oil was delivered by a Buchler Polystaltic pump as close to the propellers as possible via a piece of Tygon tubing. The emulsion

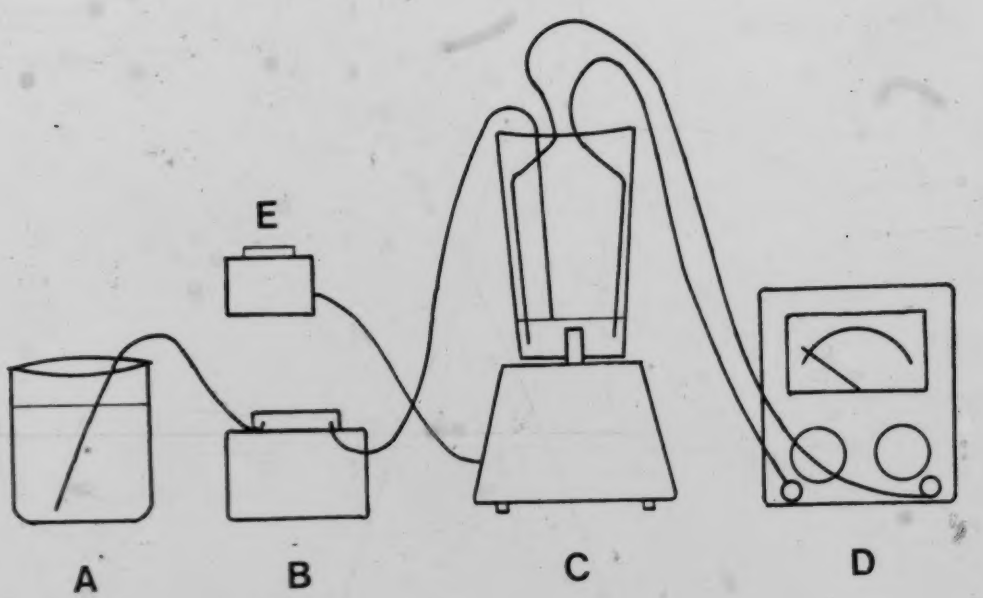

Plate 1: Experimental set up (Method 1)
A: oil supply
B: pump
C: Waring Blendor
D: ohmmeter
E: variable autotransformer

breakdown end-point was revealed by a sudden increase in resistance indicated by the Avometer 8 ohmmeter (plate 2). The experiment was performed at room temperature.

Under standard conditions, 20 ml of pH 6.0 slurry were weighed into a 600 ml beaker. After a few seconds of mixing at a rate of 1 200 rpm, the addition of rapeseed oil was started at a rate of 20 ml/min until the emulsion broke.

Method III

The experimental set up is the same in the preceding method except for the mixer and the propeller. A Fisher Stedi Speed stirrer was used to form the emulsion equipped with a four-bladed propeller. The speed of the mixer was set at 800 rpm using the control unit provided (plate 3). The rest of the procedure was exactly the same as for Method II.

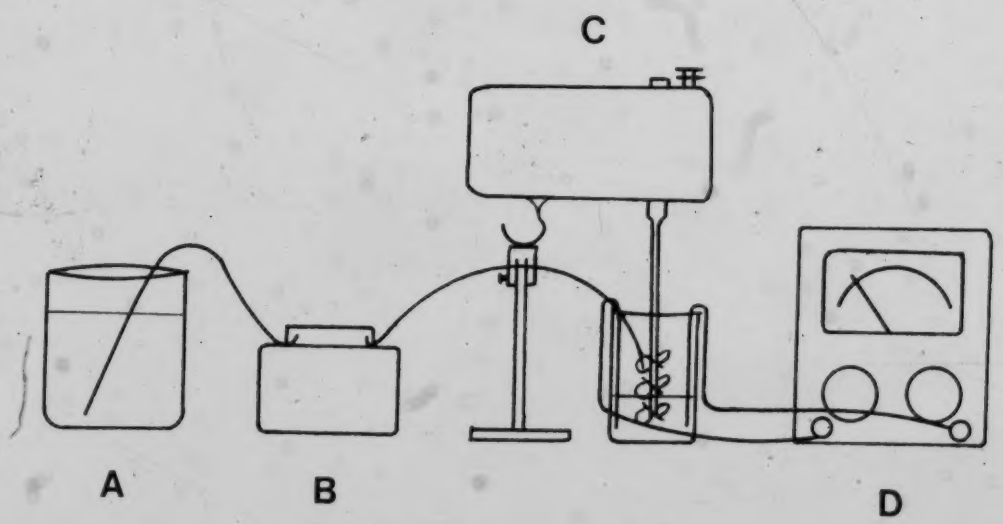

Plate 2: Experimental set up (Method II)
A: oil supply
B: pump
C: Lightnin stirrer
D: ohmmeter

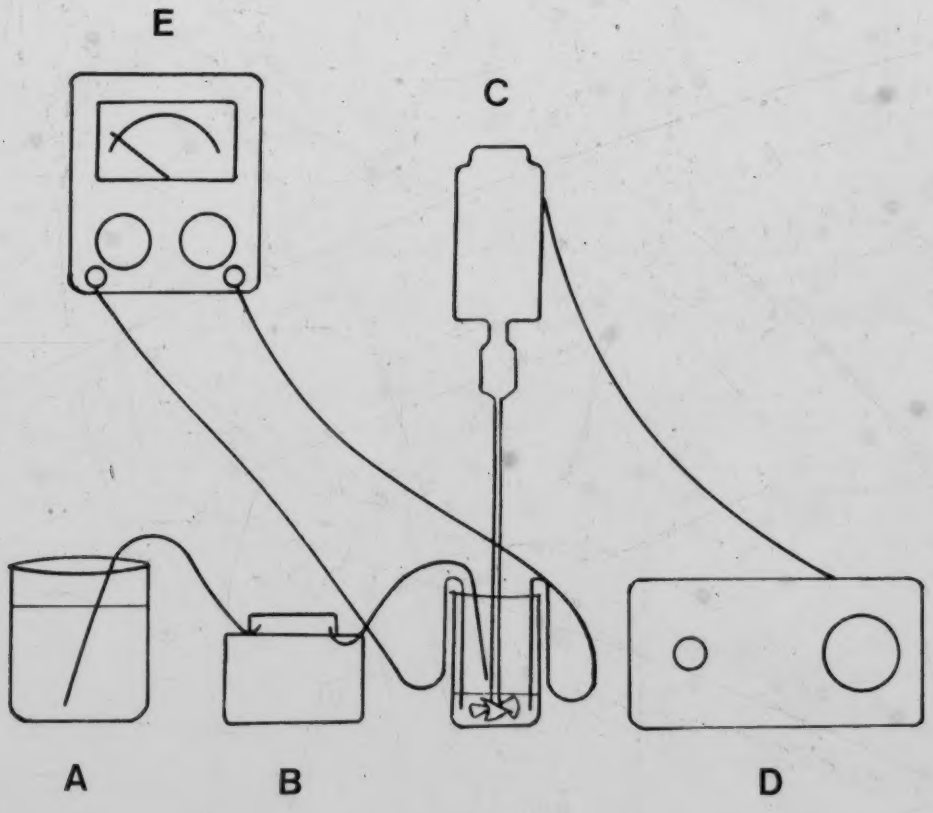

Plate 3: Experimental set up (Method III)
A: oil supply
B: pump
C: Stedi Speed stirrer (motor)
D: Stedi Speed stirrer (control unit)
E: ohmmeter

RESULTS AND DISCUSSION

Method I

Preliminary experiments

Preliminary experiments, not reported here, in which the EC was measured at a protein level of 0,02 g/g slurry, revealed no difference in EC between skeletal and cardiac muscles; as well, no increase in EC was found for skeletal muscle with time of extraction in 0,6 M NaCl solution (2°C) up to 24 hours. It was reported by Carpenter and Saffle (1964) that the EC of the cardiac muscle was lower than that of the skeletal muscle (table 11, from Acton and Saffle 1972). Borton *et al.* (1968) stated that preblending of the meat with the salt greatly enhanced its EC. Therefore, the method had to be evaluated to ensure that it was capable of detecting differences in EC.

Comparison of different kinds of proteins

In order to evaluate the method, the EC of different kinds of proteins was determined. The results of these experiments are presented in table 12. Because of a different protein concentration, the EC of butter-

TABLE 12. EC of different kinds of protein by Method I

Protein source	Protein concentration	Oil/slurry	Average oil/slurry	EC	Average EC
	g/g slurry	g/g	g/g	g oil/mg protein	g oil/mg protein
Kidney	0,20	10,33 7,74 9,90	9,32	516 387 503	469
Buttermilk powder	0,008	9,55 9,53	9,54	1 194 1 192	1 193
Papain treated [1] skeletal muscle	0,20	10,74 9,58	10,16	537 479	508

[1] 0,02% enzyme on a slurry basis (0,02 g protein/g slurry)
pH 6.0, 2°C, with agitation, 2 hours

milk powder was much higher than that of the other protein slurries. However, it must be noted that the amount of oil emulsified was similar in each titration being around 9,6 g oil/g slurry. This cast doubt on the validity of the method.

Effect of enzymatic digestion

Effect on a skeletal muscle slurry

To further check the method, the effect of enzymatic digestion was explored. It was reported by Du Bois *et al.* (1972) that the EC of bovine skeletal muscle was affected by proteolysis; during the first two hours of proteolysis, the EC decreased after which it increased and then subsequently decreased, leveling after 8 hours. In all cases, however, the values of EC were within a range from 50 to 56 ml oil/200 mg protein. Our results are reported in table 13. Results showed a steady decrease in EC during the first four hours of proteolysis which was a good indicator that the method was sufficiently sensitive to detect changes in EC.

TABLE 13. Effect of enzymatic digestion on EC of skeletal muscle by Method I [1]

Time	Oil/slurry	EC
hours	g/g	g oil/g protein
0	9,55	477
1	9,34	467
2	9,02	451
4	8,27	413

[1] 0,02% enzyme on a slurry basis (0,02 g protein/g slurry) pH 6.0, room temperature, under agitation

Comparison between skeletal and cardiac muscle slurries

The effect of enzymatic digestion was then further investigated in order to compare the behaviour of skeletal and cardiac muscles under similar conditions. The results are presented in table 14 for skeletal muscle and table 15 for cardiac muscle. No effect of the enzyme on the EC of the muscle slurries was found in either case, values of EC exhibiting large variations but no trend. It must be noted however that the EC of the cardiac muscle slurry was generally higher than that of the skeletal muscle slurry.

Effect of protein concentration on EC

Whereas the EC expressed as volume of oil per unit of protein was shown to decrease as the protein concentration was increased, the amount of oil emulsified increased with protein concentration (Acton and Saffle 1972; Gillett *et al.* 1977). This relationship between EC and protein concentration was true for many different systems; therefore, the effect of protein concentration was investigated under the conditions of the present study. A similar trend was observed between EC and protein concentration for buttermilk powder, skeletal and cardiac muscle slurries. The results are presented in the next sections.

Buttermilk powder

The effect of protein concentration on the EC of a buttermilk powder solution is presented in table 16. Curves obtained from these results show the characteristic negative curvilinear relationship between EC and protein

TABLE 14. Effect of enzymatic digestion on EC of skeletal muscle by Method I [1]

Time	Oil/slurry	EC
hours	g/g	g oil/g protein
2	10,77	291
6	9,70	262
24	13,04	352
30	10,69	289
53	9,22	249

[1] 0,2% enzyme on a slurry basis (0,037 g protein/g slurry) pH 6.0, 2°C, without agitation

TABLE 15. Effect of enzymatic digestion on EC of cardiac muscle by Method I [1]

Time	Oil/slurry	EC
hours	g/g	g/g protein
5	12,28	315
18	12,80	328
22	12,15	312
43	15,17	389
48	15,46	396
66	14,17	363

[1] 0,2% enzyme on a slurry basis (0,039 g protein/g slurry) pH 6.0, 2°C, without agitation

concentration and a positive linear relationship when the amount of oil emulsified is considered (figure 13). The value of EC for the buttermilk is different from the one obtained in the first experiment comparing the

TABLE 16. Effect of protein concentration on EC by Method I (buttermilk powder)

Protein concentration	Oil/solution	EC
g/g solution	g/g	g oil/g protein
0,02	5,81	291
0,04	6,99	175
0,08	7,50	94
0,1	8,08	81

EC of different kinds of proteins. This discrepancy can be attributed in part to the fact that different protein levels are being compared, since EC rises sharply at low protein concentrations. But the difference in protein levels cannot account for the large difference in EC leaving doubt on the reliability of the method.

Skeletal and cardiac muscle slurries

Values of EC obtained for skeletal muscle slurries of different protein concentration at two different times of extraction in 0,6 M saline solution are presented in table 17. Results of a comparable study using cardiac muscle slurries are presented in table 18. In either case, EC decreased curvilinearly as protein concentration was increased (figures 14, 16). Most important, as can be seen from figures 14 and 16, there is still no difference between the EC of skeletal and cardiac muscle slurries either before or after preblending. When the amount of oil expressed as g oil/g slurry is considered, a

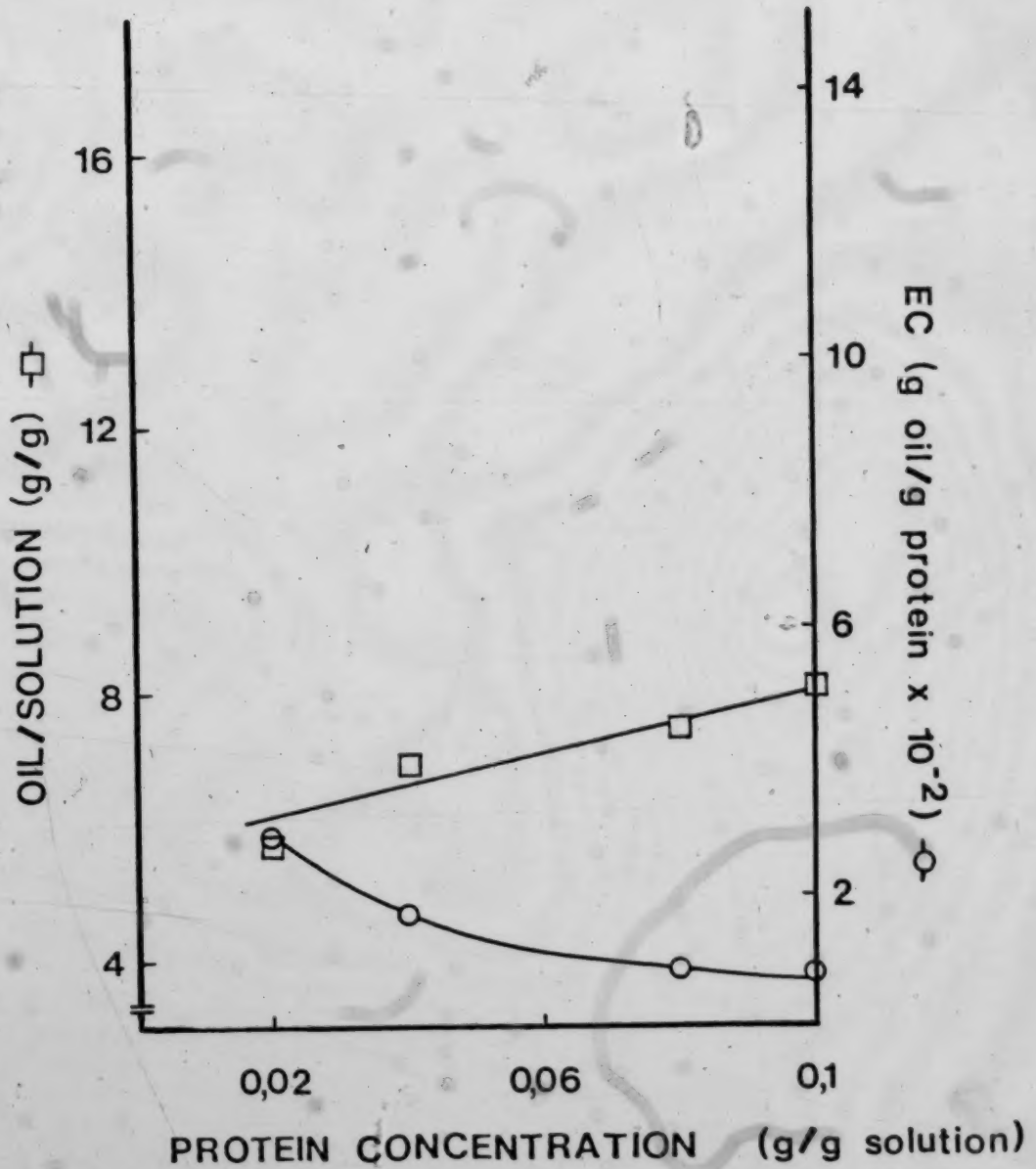

Figure 13: Effect of protein concentration on EC and the amount of oil emulsified for a buttermilk powder solution by Method I

TABLE 17. Effect of protein concentration on EC by Method I (skeletal muscle)

Protein concentration	Before preblending		After preblending	
	oil/slurry	EC	oil/slurry	EC
g/g slurry	g/g	g oil/g protein	g/g	g oil/g protein
0,005	5,97	1 194	6,63	1 326
0,01	7,63	763	7,86	786
0,02	8,97	449	9,33	467
0,03	10,95	365	10,99	366
0,04	11,87	297	10,70	268
0,06	12,85	214	13,64	227

TABLE 18. Effect of protein concentration on EC by Method I (cardiac muscle)

Protein concentration	Before preblending		After preblending	
	oil/slurry	EC	oil/slurry	EC
g/g slurry	g/g	g oil/g protein	g/g	g oil/g protein
0,004	5,19	1 297	6,37	1 593
0,008	5,97	746	6,90	863
0,015	7,06	471	7,89	526
0,03	8,68	289	11,06	369
0,04	12,95	324	15,87	397

positive relationship, sometimes linear, is obtained with respect to protein concentration, again showing no difference between skeletal and cardiac muscle slurries before or after preblending (figures 15, 17). These results were confirmed by another similar experiment. The results of

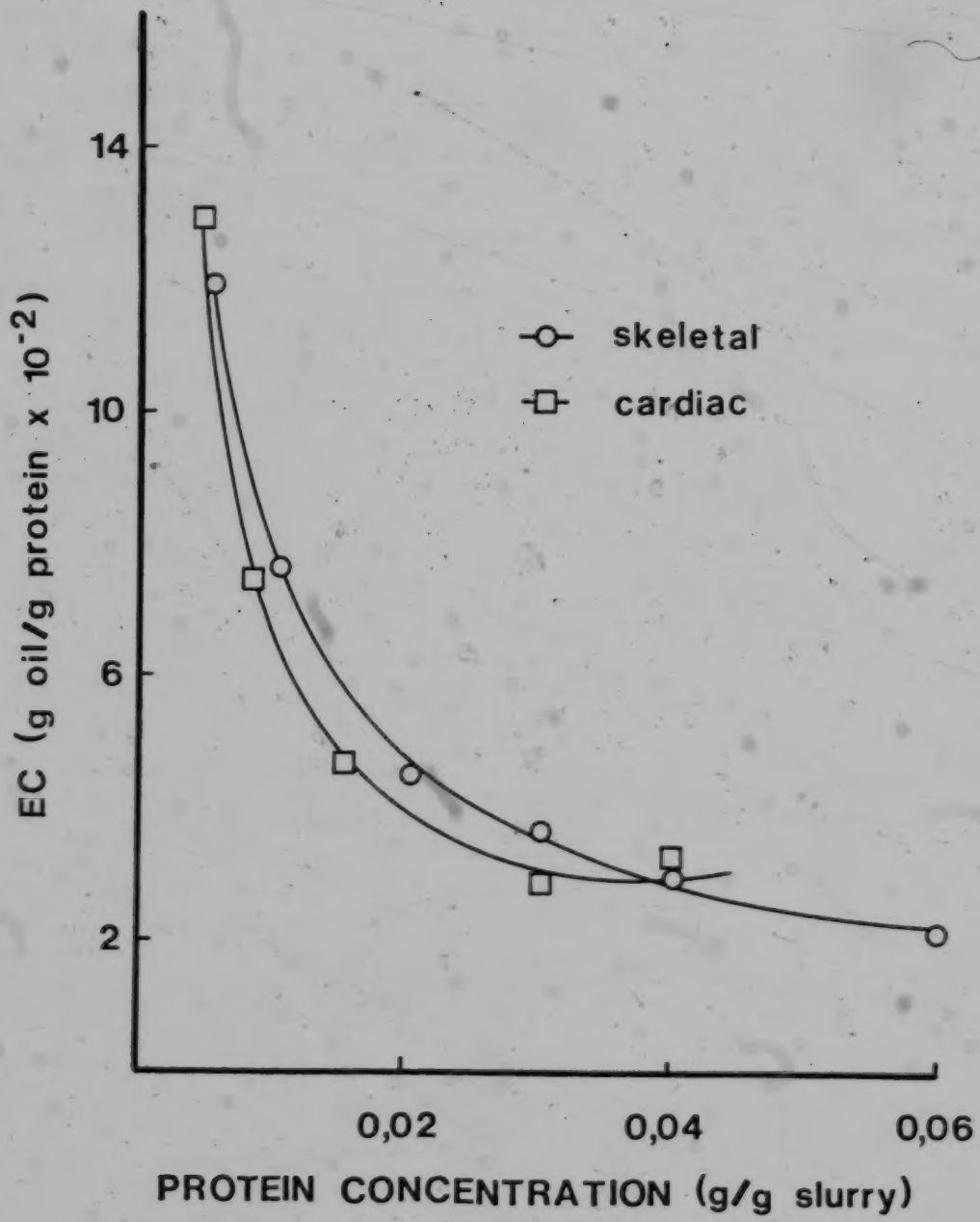

Figure 14: Effect of protein concentration on EC of skeletal and cardiac muscle slurries before preblending by Method I

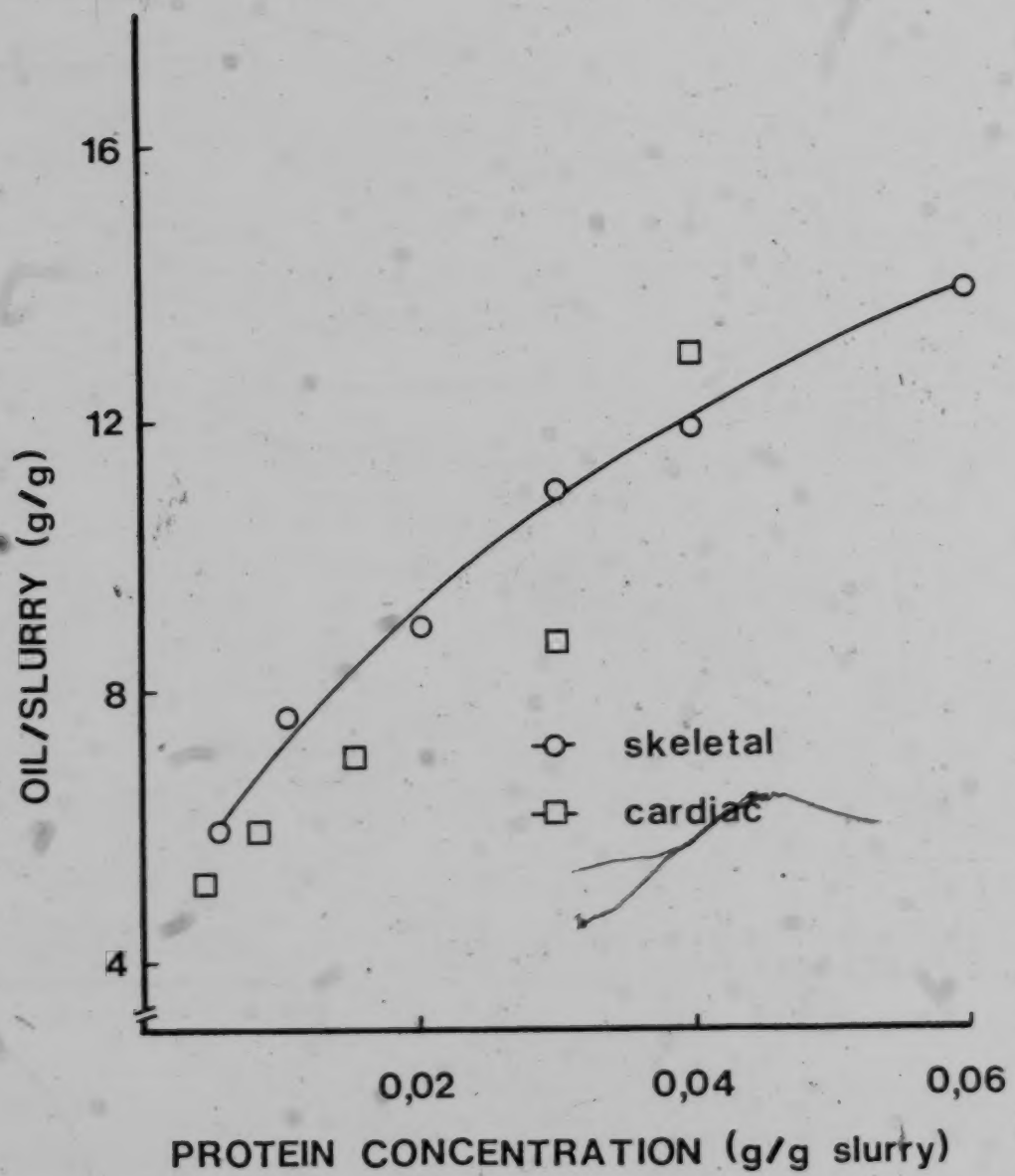

Figure 15: Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries before preblending by Method I

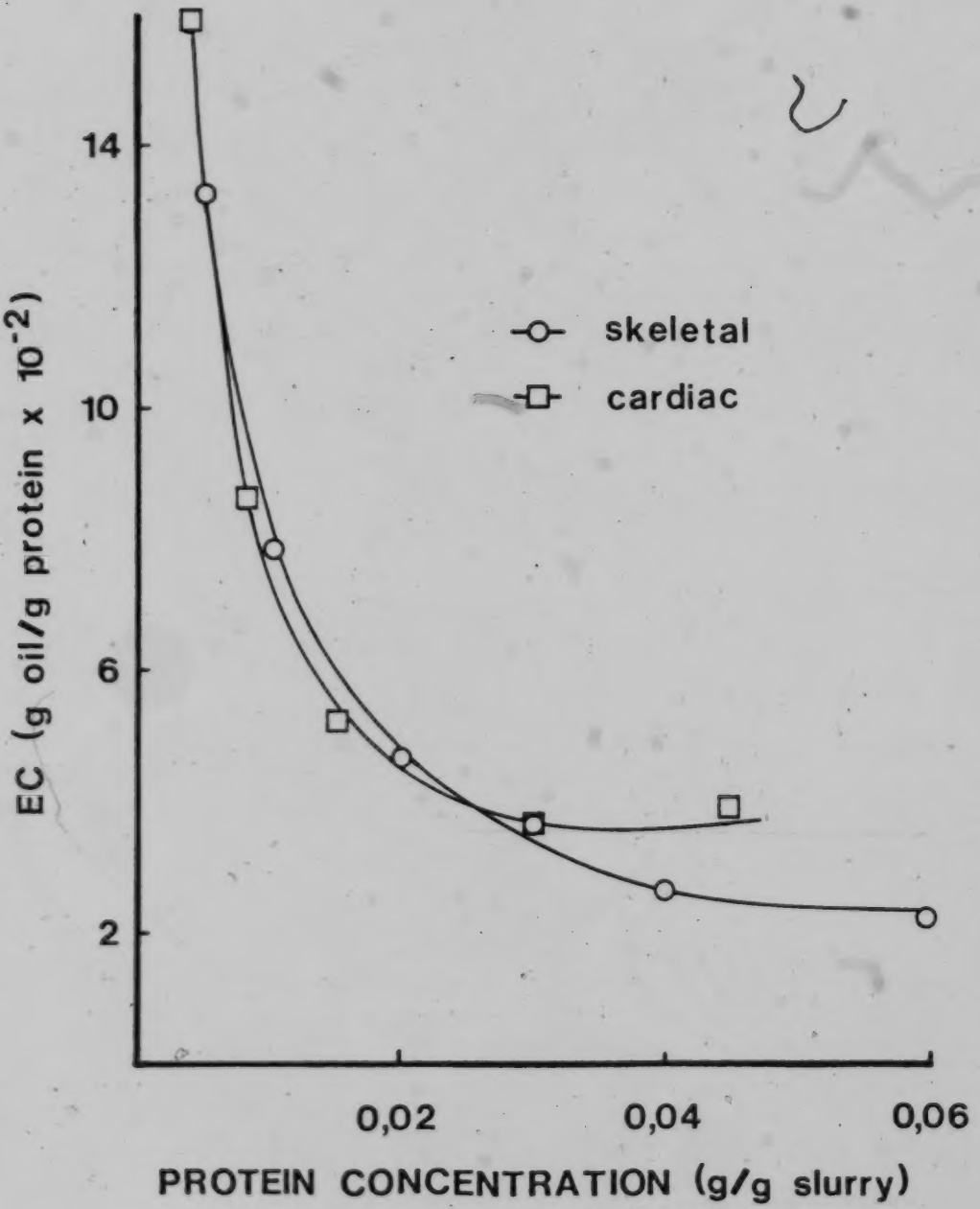

Figure 16: Effect of protein concentration on EC of skeletal and cardiac muscle slurries after preblending by Method I

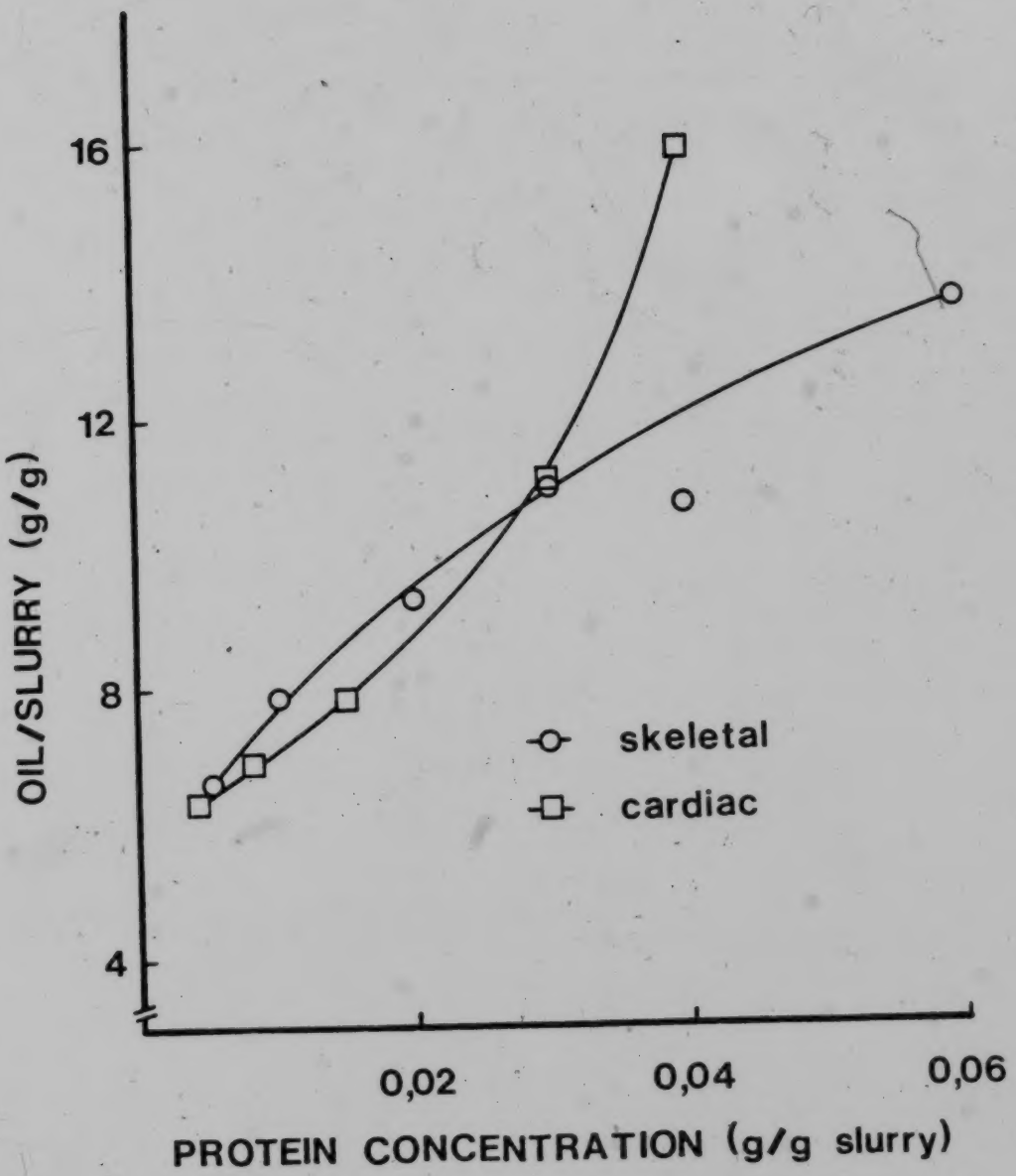

Figure 17: Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries after preblending by Method I

this second experiment are presented in tables 19 and 20 for skeletal and cardiac muscle slurries, respectively, and figures 18 and 20 for the effect of protein concentration on EC and figures 19 and 21 for the effect of protein concentration on the amount of oil emulsified.

TABLE 19. Effect of protein concentration on EC by Method I (skeletal muscle)

Protein concentration	Before preblending		After preblending	
	oil/slurry	EC	oil/slurry	EC
g/g slurry	g/g	g oil/g protein	g/g	g oil/g protein
0,011	6,72	611	7,33	666
0,016	7,55	472	7,71	482
0,021	7,91	377	8,31	396
0,031	8,63	278	9,49	306
0,037	9,78	264	10,03	271
0,041	9,70	237	11,65	284
0,061	20,31	333	11,97	196

TABLE 20. Effect of protein concentration on EC by Method I (cardiac muscle)

Protein concentration	Before preblending		After preblending	
	oil/slurry	EC	oil/slurry	EC
g/g slurry	g/g	g oil/g protein	g/g	g oil/g protein
0,009	6,96	773	6,78	753
0,014	7,39	528	7,17	512
0,018	7,73	429	7,63	424
0,027	8,86	328	9,30	344
0,035	11,32	290	12,66	325
0,039	10,78	308	10,99	314
0,052	15,56	299	14,32	275

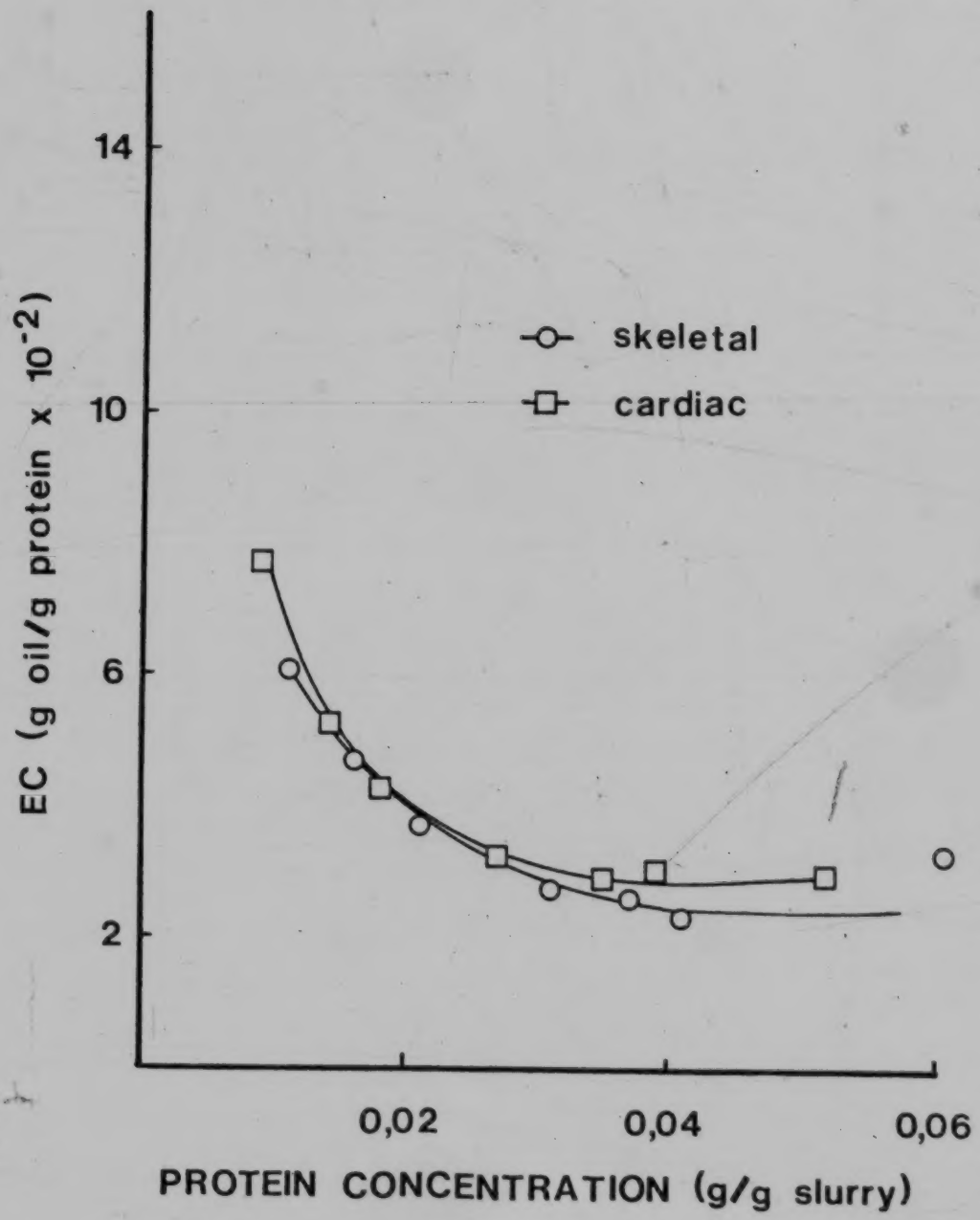

Figure 18: Effect of protein concentration on the EC of skeletal and cardiac muscle slurries before preblending by Method I

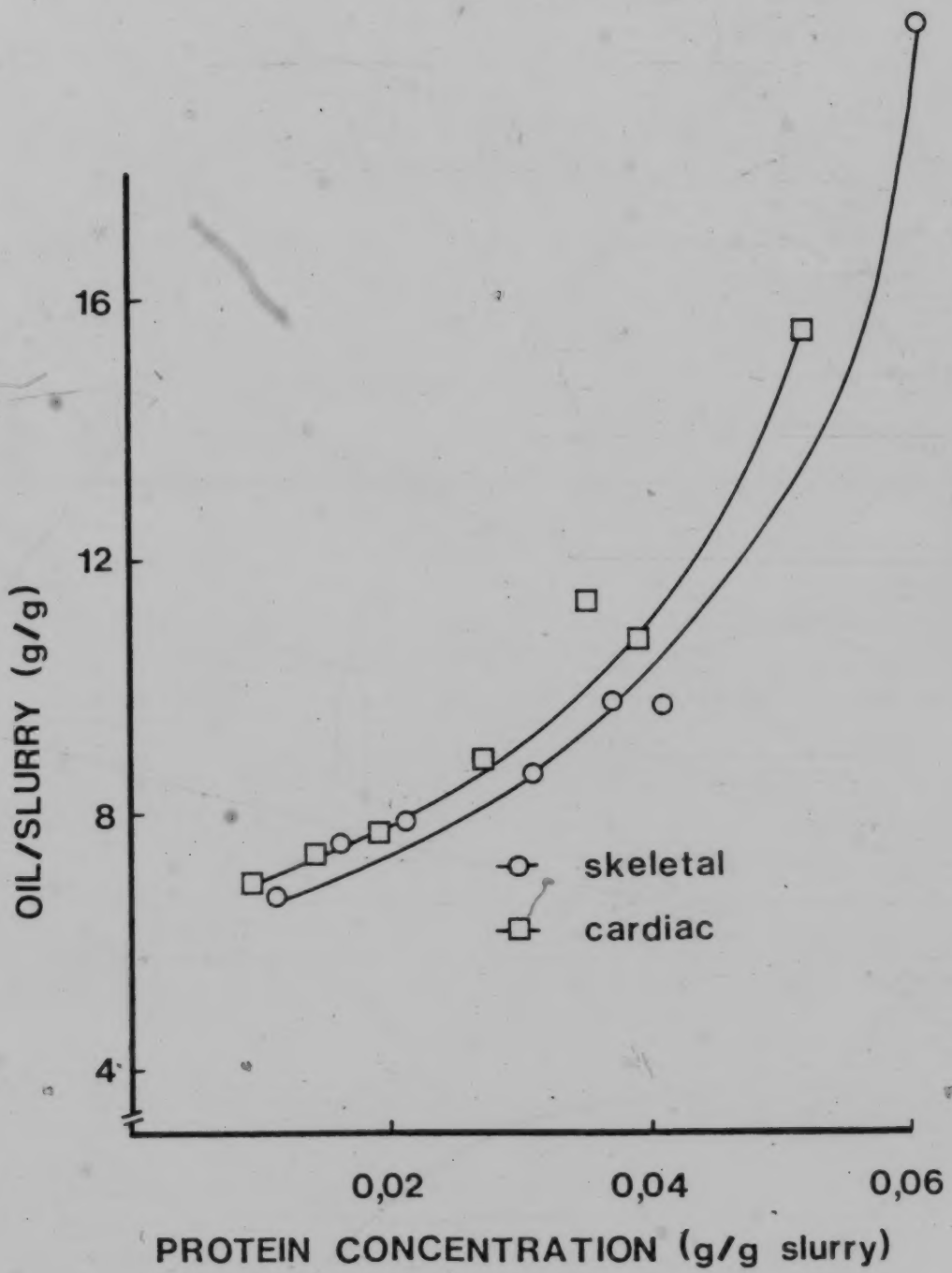

Figure 19: Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries before preblending by Method I

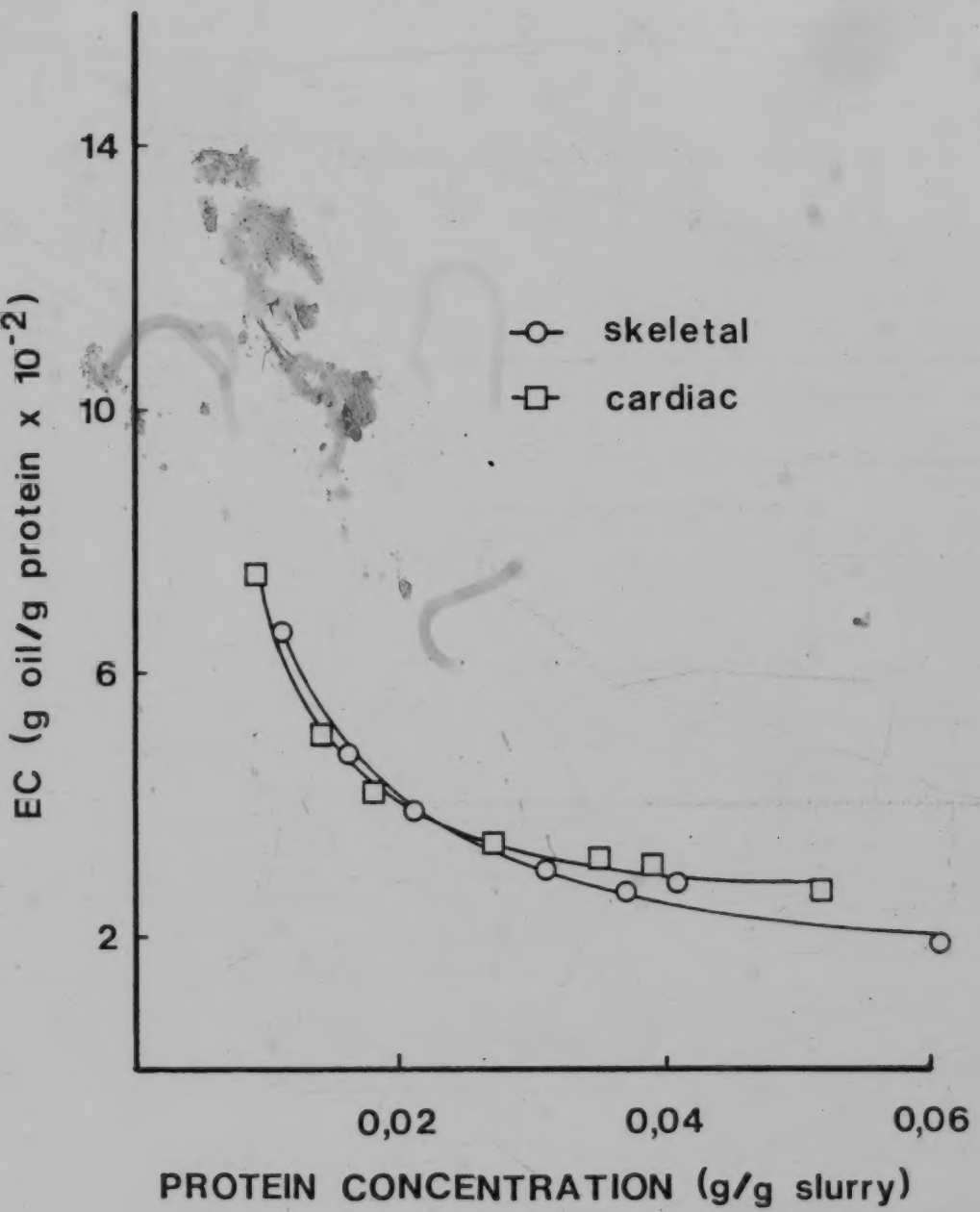

Figure 20: Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries after preblending by Method I

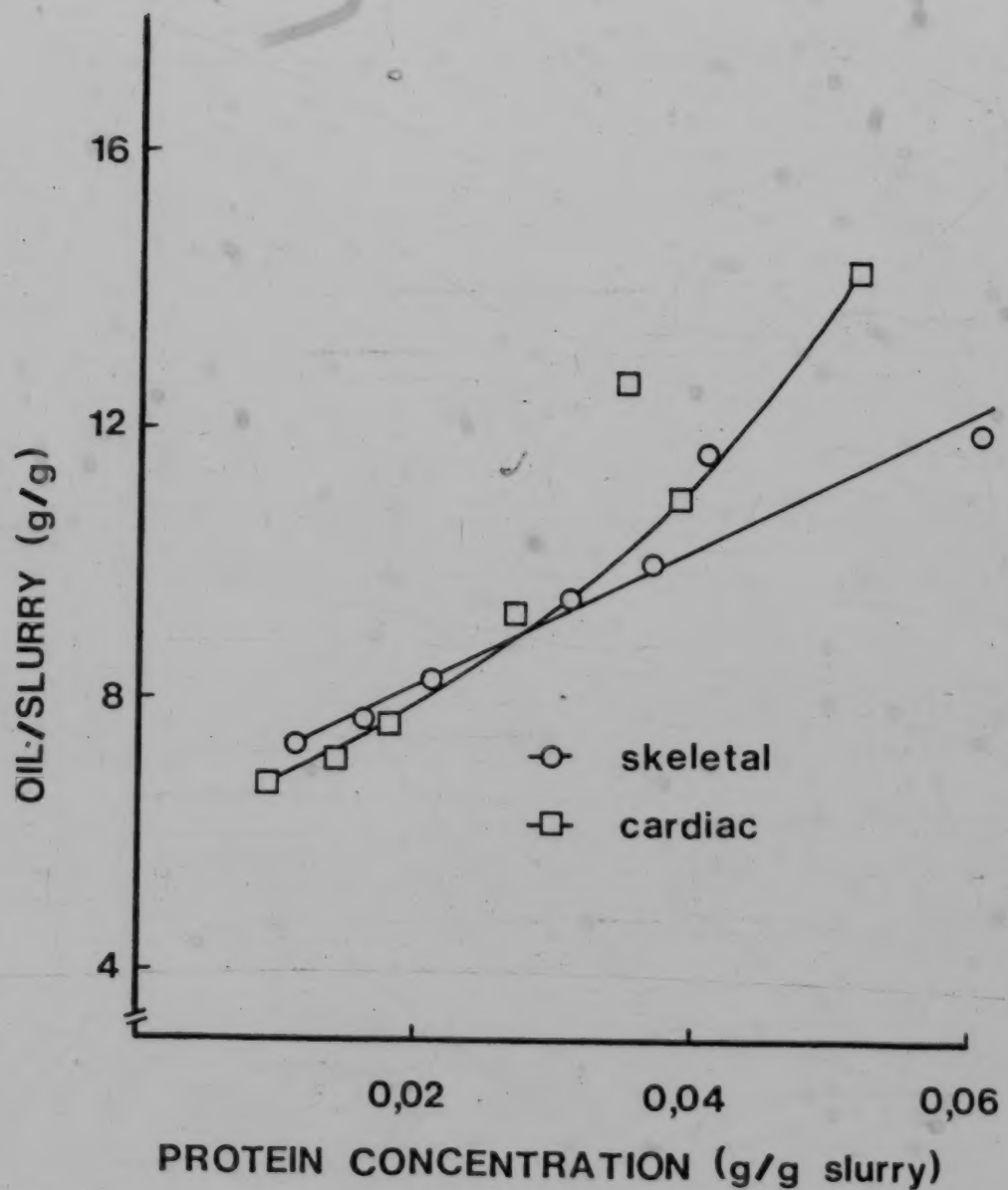

Figure 21: Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries after preblending by Method I

Sarcoplasmic and myofibrillar proteins

Since it has long been recognized that the myofibrillar or salt-soluble proteins are the principal emulsifying agent in meat, the EC of the different protein fractions was determined for both skeletal and cardiac muscles. The EC of sarcoplasmic or water-soluble proteins and that of salt-soluble proteins was determined; however, the EC of the connective tissue proteins could not be measured because these proteins were insoluble under test conditions. The results for skeletal muscle protein fractions are presented in table 21 and values for cardiac muscle protein fractions are presented in table 22. Protein fractions from cardiac muscle are slightly more efficient at the same protein level and the myofibrillar proteins are superior to the sarcoplasmic proteins. Swift *et al.* (1961) reported that the efficiency of the salt-soluble proteins varied with their concentration whereas that of the water-soluble proteins was not affected. On the other hand, Tsai *et al.* (1972) found the EC of both protein fractions to vary with protein concentration (figure 1, from Tsai *et al.* 1972). Although more data would be required, the results presented in tables 21 and 22 indicate a stronger influence of protein concentration in the case of the myofibrillar proteins.

Effect of preblending

Borton *et al.* (1968) reported that chopping meat and salt together and allowing time for extraction of the salt-soluble proteins (preblending) greatly enhanced the EC of the meat. The effect of preblending was presented in a previous section on the effect of protein concentration

TABLE 21. EC of skeletal muscle protein fractions by Method I

Protein concentration	Sarcoplasmic proteins		Myofibrillar proteins	
	oil/slurry	EC	oil/slurry	EC
g/g slurry ($X10^3$)	g/g	g oil/g protein	g/g	g oil/g protein
13,0	--	---	7,78	598
12,7	6,67	494	--	---
5,7	5,53	970	--	---
4,9	--	---	9,44	1 942

TABLE 22. EC of cardiac muscle protein fractions by Method I

Protein concentration	Sarcoplasmic proteins		Myofibrillar proteins	
	oil/slurry	EC	oil/slurry	EC
g/g slurry ($X10^3$)	g/g	g oil/g protein	g/g	g oil/g protein
14,4	5,45	517	--	---
13,0	7,72	594	9,63	741
9,6	--	---	18,30	1 906
7,2	5,90	819	9,40	1 306
4,8	--	---	8,14	1 696
3.6	--	---	5,63	1 564

where it was concluded that the EC of skeletal and cardiac muscle slurries was similar before and after preblending. From tables 17 and 19 presenting data for skeletal muscle slurries, it can be seen that only a small increase in EC was observed after preblending. The situation was the same for cardiac muscle slurries as reported in tables 18 and 20.

Effect of pH

The capacity of a protein to form a film is greatest at its isoelectric point because the number of salt bridges and hydrogen bonds is maximum at that particular pH (Schut 1976). The effect of pH was investigated using skeletal and cardiac muscle slurries. The results are presented in table 23. No influence of pH was found for the skeletal muscle slurry whereas the cardiac muscle slurry there was a trend towards steady increase of both the EC and the amount of oil emulsified with increasing pH.

TABLE 23. Effect of pH on the EC of skeletal and cardiac muscle slurries by Method I

pH	Skeletal muscle [1]		Cardiac muscle [2]	
	oil/slurry	EC	oil/slurry	EC
	g/g	g oil/g protein	g/g	g oil/g protein
5.0	11,73	317	9,67	248
5.5	10,09	273	10,79	277
6.0	11,66	315	11,29	259
6.5	12,68	343	11,42	293
7.0	11,56	312	12,23	314

[1] Protein level: 0,037 g/g

[2] Protein level: 0,039 g/g

Problems associated with Method I

Using the Waring Blendor technique, many problems associated with the determination of the end-point were encountered. The method of Marshall *et al.* (1975) using the increased visibility of coloured oil droplets to determine the end-point revealed no change in the appearance of the emulsion during the titration, when observed through the sides of the Waring Blendor jar. This phenomenon was probably caused by the high speed cutting-mixing action of the blades. On the other hand, at lower speeds of mixing, emulsions stopped the action of the blades. Therefore, the end-point was determined by measuring the electrical resistance with an ohmmeter.

In my early attempts to measure the EC of meat proteins using the Waring Blendor method, a large number of trials were carried out unsuccessfully because it was impossible to determine the oil titration end-point using the ohmmeter. Subsequently, more reliable responses were obtained and experiments could be started and finished. However, every now and again, the system for determining the end-point failed for no obvious reason. Even when the response was considered to be good, it never was as good as the response obtained by Webb *et al.* (1970). These researchers observed a very sharp increase in the electrical resistance going from a few ohms to infinity. In our laboratory, using the Waring Blendor method, the resistance increased but never reached infinity; this behaviour cast some doubt as to whether or not this resistance change represented the actual titration end-point. Such problems in determining the end-point were encoutered throughout the whole series of experiments with the Waring Blendor and were believed to be due to the very efficient mixing action of the blades.

The type of mixing could be responsible for the insensitivity of the method. In general, no large variation in EC values and in amounts of oil added was observed except when the protein concentration was changed and when the EC of the extracted muscle protein fractions was measured. Further evidence of the effect of the type of mixing was provided when experiments using a propeller were started. The viscosity of the emulsions obtained from the two systems was totally different; very thick emulsions sometimes resistant to mixing were obtained using the propeller while emulsions of very low viscosity were formed with the Waring Blendor. In order to solve the previously mentioned problems, another type of mixing action was investigated: the propeller or Method II.

Method II

Preliminary experiments

The first experiments were attempts to reproduce the experiments conducted by Borton *et al.* (1968), Swift *et al.* (1961) and Webb *et al.* (1970). In all three cases, the same problem of inadequate mixing was encountered. To solve that problem three propellers were stacked on top of each other on the same shaft in the method of Borton *et al.* (1968). In order to evaluate this new system, myofibrils were extracted and the EC was measured under various conditions. However, it was necessary to establish standard conditions before this evaluation could be carried out. The next sections deal with the experiments that were done in order to establish the standard conditions.

Effect of salt

A blank sample consisting of a salt solution alone for both NaCl and KCl was titrated and the values obtained for both salts were relatively high. Consequently, the effect of salt concentration was investigated for concentrations ranging from 0,15 to 2,0 M. The experiment was repeated three times for each salt as reported in table 24 for NaCl and table 25 for KCl. Although the level of oil added was relatively high for both NaCl and KCl solutions the effect of salt concentration was small.

TABLE 24. Effect of NaCl concentration on the amount of oil added to a salt solution by Method II

Salt concentration	Oil/solution			Average [1] oil/solution			Average [2] oil/solution
M	g/g			g/g			g/g
trial	1	2	3	1	2	3	
0,15	4,44	3,95	3,87	4,38	3,35	3,87	3,87
	4,21	2,88	--				
	4,50	3,21	--				
0,6	3,05	2,33	2,61	2,70	2,92	2,17	2,60
	3,04	3,14	1,72				
	2,01	3,28	--				
1,0	2,65	2,65	2,95	3,22	2,55	2,32	2,70
	3,60	2,47	1,68				
	3,41	2,54	--				
2,0	3,81	2,94	2,79	3,62	2,89	2,91	3,14
	3,59	2,45	3,03				
	3,47	3,27	--				

[1] This value represents the average of the three values of the corresponding trial

[2] This value represents the average of the average values in [1] for the three trials

Effect of buffer

Another blank containing only the buffer in which the myofibrils were suspended was titrated with oil and the volume of oil added was relatively large. Therefore, the effect of the buffer on the amount of oil added was studied by varying the concentration of buffer in a 50 ml aliquot hence diluting it. Results clearly showed that the amount of oil added decreased as the buffer was diluted (table 26, figure 22). This effect of buffer concentration on the amount of oil

TABLE 25. Effect of KCl concentration on the amount of oil added to a salt solution by Method II

Salt concentration	Oil/solution			Average [1] oil/solution			Average [2] oil/solution
M	g/g			g/g			g/g
trial	1	2	3	1	2	3	
0,15	4,03	6,43 [3]	3,81				
	3,78	2,99	3,01	3,91	2,99	3,24	3,38
	--	--	2,90				
0,6	2,87	2,39	2,72				
	3,03	2,32	2,11	2,97	2,36	2,41	2,58
	3,02	--	2,41				
1,0	3,11	3,10	2,64				
	3,14	--	1,94	3,09	3,10	2,24	2,81
	3,03	--	2,13				
2,0	2,91	2,04	0,85 [3]				
	2,69	3,07	1,98	2,92	2,56	2,18	2,55
	3,71	--	2,48				

[1] This value represents the average of the three values of the corresponding trial

[2] This value represents the average of the average values in [1] for the three trials

[3] Discarded values

added could not be attributed to the salt because salt concentration was found to have no effect on the amount of oil added. If one compares results from tables 25 and 26 it becomes evident that tris has an effect on EC since the value of EC for salt alone at a concentration of 0,15 M is doubled in the presence of tris. In an attempt to measure the effect of tris alone, the very high resistance of the initial solution made it impossible to determine the end-point; consequently, it was impossible to determine the effect of tris concentration on EC.

TABLE 26. Effect of buffer concentration on the amount of oil added to a buffer solution by Method II

Tris concentration	KCl concentration	Oil/solution	Average oil/solution
M	M	g/g	g/g
0,006	0,3	1,61 2,50	2,06
0,012	0,06	5,12 4,08	4,60
0,018	0,09	4,60 6,60	5,60
0,024	0,12	4,98 5,90	5,44
0,03	0,15	6,89	6,89

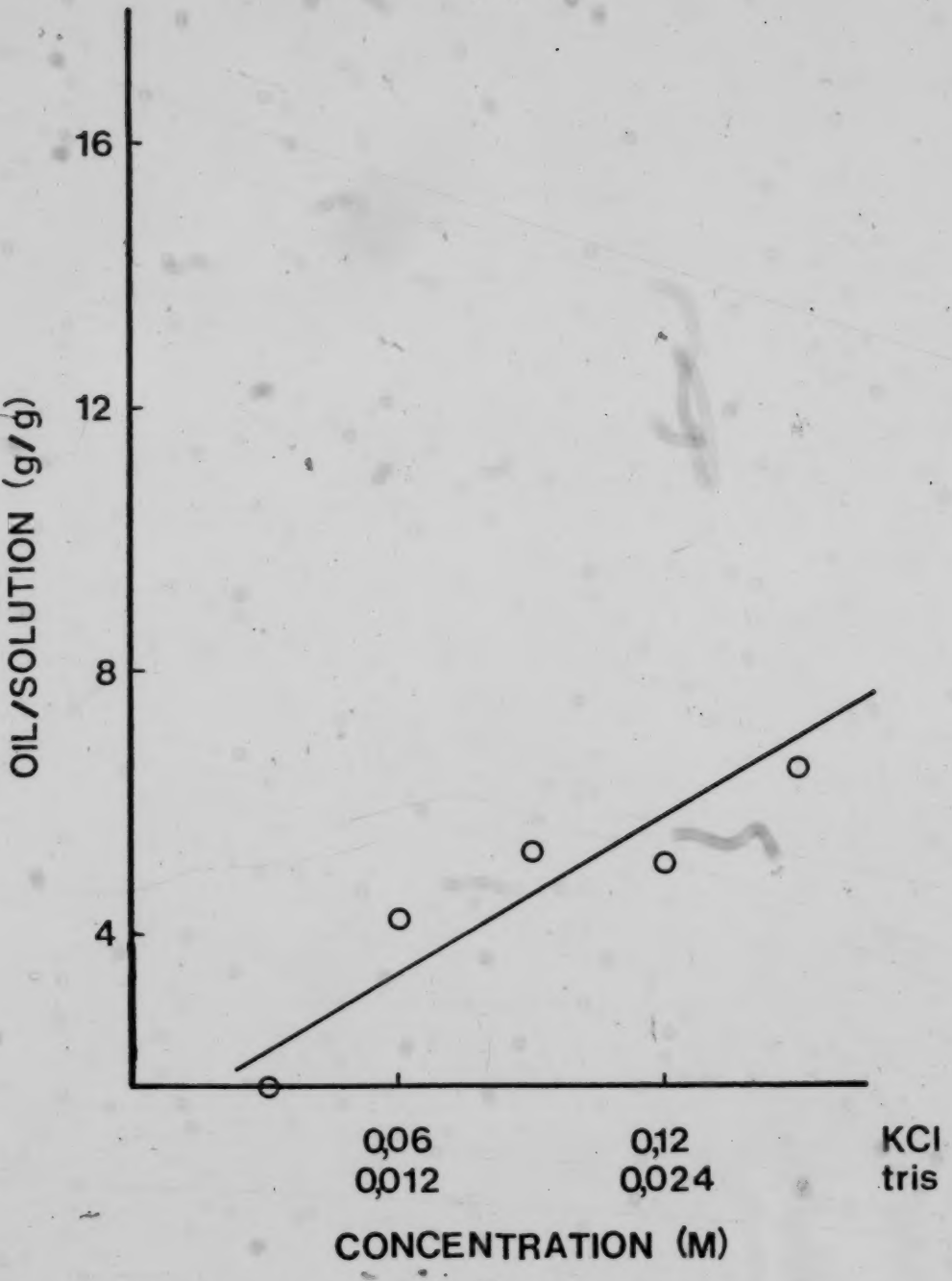

Figure 22: Effect of buffer concentration on the amount of oil added to a KCl-tris buffer by Method II

Effect of rate of oil addition

Reports on the effect of the rate of oil addition are contradictory; Swift *et al.* (1961) found a positive linear relationship between the rate of oil addition and the amount of oil emulsified whereas Carpenter and Saffle (1964) found no effect under controlled conditions. In order to determine the effect of the rate of oil addition on the amount of oil added in the present system, three experiments were conducted using a 1,0 M NaCl solution. The results are presented in table 27 and curves obtained from these results are presented in figure 23. There was a wide variation in the values of amount of oil added making it difficult to draw any conclusion concerning the effect of rate of oil addition in a 1,0 M NaCl solution. There is a trend towards increasing volume of oil with increasing rate of oil addition. A rate of 20 ml/min was chosen for further experiments because a reasonable amount of oil was required. At a rate of 20 ml/min, a value of approximately 3,6 g oil/g slurry was found which compared well with the value of 2,7 g oil/g slurry reported for a 1,0 M NaCl solution in the study of the effect of salt concentration reported previously (table 24).

Effect of pH

Using a 1,0 M NaCl solution again, the effect of pH on the amount of oil added was investigated. The pH was found to have no effect (table 28).

TABLE 27. Effect of rate of oil addition on the amount of oil added to a 1,0 M NaCl solution by Method II

Rate	Oil/solution	Average oil/solution	Rate [1]	Oil/solution	Average oil/solution	Rate	Oil/solution	Average oil/solution
ml/min	g/g	g/g	ml/min	g/g	g/g	ml/min	g/g	g/g
	2,87			2,32			2,42	
5	1,22	1,93	7,5	1,18	1,63	8	1,69	2,26
	1,70			1,40			2,69	
	5,11			1,89			4,26	
11,5	6,47	5,69	18	2,33	2,04	18	4,13	4,02
	5,48			1,91			3,67	
	5,37			2,71			6,53	
18	5,03	4,82	32	3,01	2,75	30	5,20	5,69
	4,06			2,53			5,34	
	4,56			4,34			5,79	
25	5,40	4,89	47	4,24	4,29	39	6,44	6,32
	4,72			--			6,72	
	5,33			6,56			3,34	
38	2,37	3,55	52	5,40	5,98	45	1,45	3,24
	2,96			--			4,94	

[1] Speed of mixing 1 500 rpm

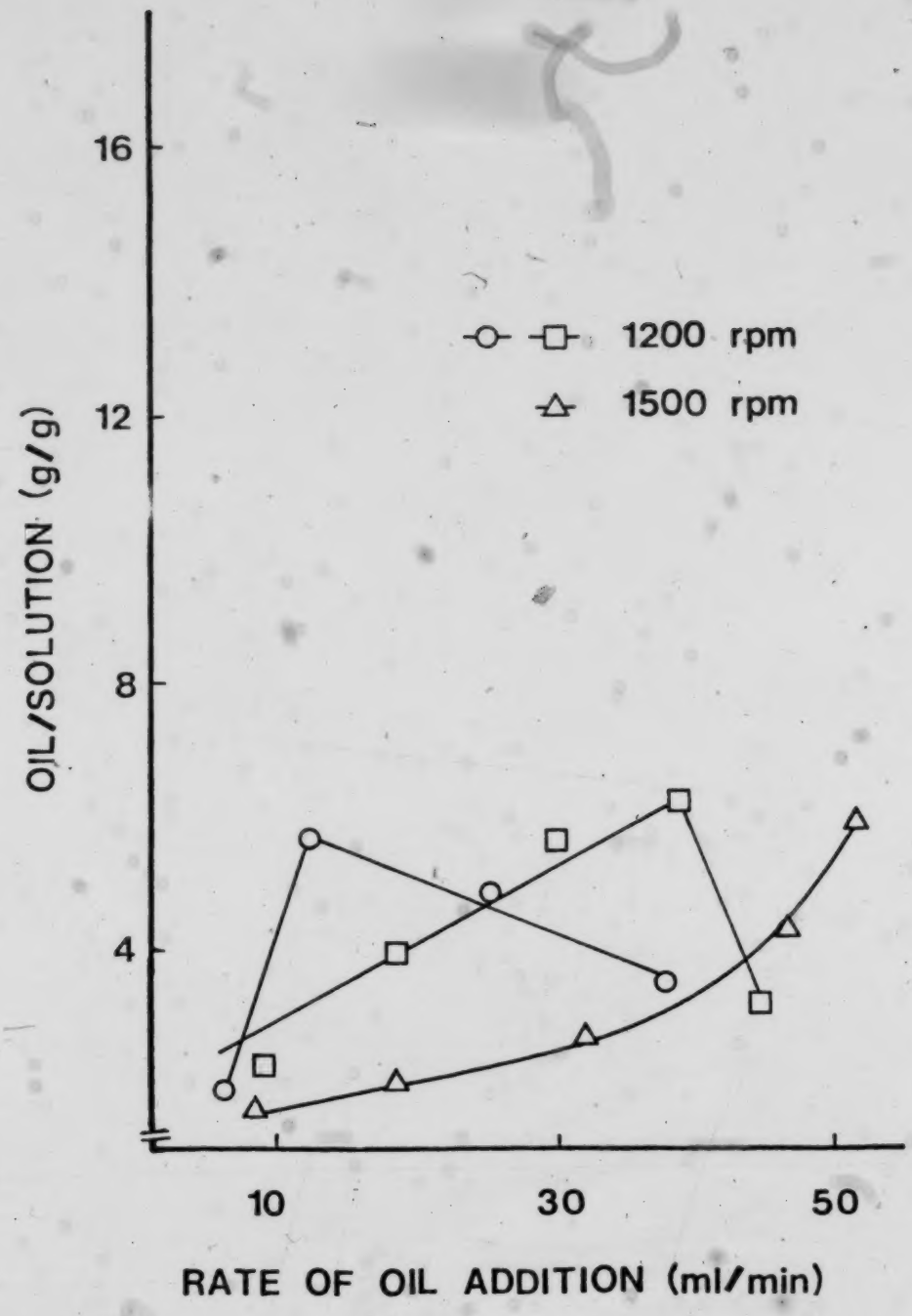

Figure 23: Effect of rate of oil addition on the amount of oil added to a 1,0 M NaCl solution by Method II

TABLE 28. Effect of pH on the amount of oil added to a 1,0 M NaCl solution by Method II

pH	Oil/solution	Average oil/solution
	g/g	g/g
5.0	3,69 3,74 3,39	3,61
5.5	3,25 2,35 3,48	3,03
6.0	3,48 3,47 2,79	3,25
6.5	3,84 3,35 3,49	3,56
7.0	2,81 3,16 3,06	3,01
7.5	2,93 3,40 3,70	3,34
8.0	3,30 3,19 3,30	3,26

Effect of speed of mixing

According to the literature, the speed of mixing inversely affects the EC. The effect of speed of mixing was reported by several researchers, for example by Swift *et al.* (1961) and Carpenter and Saffle

(1964). The effect of speed of mixing was investigated using solutions of myofibrils prepared by resuspending 4 g of centrifuged myofibrils extracted from skeletal muscle into 100 ml of buffer. The EC was measured at different speeds of mixing in duplicates using 50 ml of solution at a time. The speed of mixing was also found to have an effect here as indicated by the results presented in table 29, although no trend could be identified. Less oil could be added at high speed of mixing and the nature of the emulsion was also different, its grain becoming finer as the speed of mixing was increased. This was similar to what was reported by Swift *et al.* (1961) (table 4, from Swift *et al.* 1961). However, more results would be required to ascertain this statement in the case of the present experiment. Most important is the large variation in the results. This situation was expected from the difficulty of adjusting the speed and keeping it constant in the laboratory. This lack of speed control could also explain the variation in the results obtained from the study on the rate of oil addition (figure 23).

TABLE 29. Effect of speed of mixing on the EC of a myofibril solution by Method II

Speed of mixing	Oil/solution	Average oil/solution	EC	Average EC
rpm	g/g	g/g	g oil/g myofibrils	
500	6,40 5,45	5,93	160,16 136,66	148,41
800	3,40 5,56	4,48	85,29 138,91	112,10
1 000	6,19 3,40	4,80	154,77 85,05	119,91

[1] 50 ml of a suspension prepared with 4 g of myofibrils in 100 ml buffer

Effect of initial volume of solution

The effect of the initial volume of solution was studied using a skeletal myofibril suspension and a cardiac muscle slurry. Solutions of myofibrils were prepared by resuspending 4 g of centrifuged myofibrils into 100 ml of buffer. Increasing volumes of buffer were added to 20 ml of that protein suspension so that the amount of protein was constant while the initial volume of solution increased. In the case of the meat slurry, 20 ml of a slurry containing 0,34 mg protein/g were used. Results of this experiment are presented in table 30. It was found that the initial volume of solution had an influence on the EC, a fact that was also reported by Carpenter and Saffle (1964) (figure 5, from Carpenter and Saffle 1964). These researchers observed a positive linear relationship between the volume of oil added and the initial volume of solution. In the present study, while EC was found to increase with the initial

TABLE 30. Effect of initial volume of solution on the EC of a myofibril solution and a cardiac muscle slurry by Method II

Initial volume	Myofibril solution [1]		Meat slurry [2]	
	Oil/solution	EC	Oil/slurry	EC
ml	g/g	g oil/g myofibrils	g/g	g oil/g protein
20	5,17	124,05	4,26	250
30	5,45	195,64	3,52	316
40	--	---	3,71	442
50	3,70	231,78	3,69	547

[1] 20 ml of a solution prepared with 4 g myofibrils in 100 ml buffer

[2] 20 ml of a meat slurry containing 0,34 mg protein/g

volume of solution for both myofibrils and the meat slurry, no definite effect was observed with respect to amount of oil emulsified (figures 24, 25). A standard initial volume of 20 ml was chosen for further experiments.

Effect of protein concentration

The effect of protein concentration was studied using myofibrils extracted from skeletal muscle. A suspension of myofibrils was centrifuged; the different protein concentrations correspond to increasing weight of the pellet resuspended in 100 ml of buffer. The EC was measured in duplicates using 50 ml of suspension for each determination. The results appear in table 31 from which figure 26 was obtained. It is interesting to note that the largest amount of oil was emulsified by the buffer alone, this amount being in agreement with the volume of oil emulsified by the buffer in the study on the effect of buffer. The addition of a small quantity of protein greatly reduced the amount of oil required for the emulsion to break; then, as the amount of myofibrils increased, the amount of oil emulsified also increased. The same negative curvilinear relationship was found between EC and protein concentration. The components of the buffer certainly have an effect on the EC since the amount of oil added increased when their concentration was decreased (table 26, figure 22). However, this effect seems to be minimized in the presence of protein. These two phenomena i.e. titration of a buffer solution and titration of a protein solution are different but the method of the electrical resistance fails to differentiate them. In the case of the buffer alone, it seems that one of the ingredients, tris, acts as an emulsifier, since the amount of

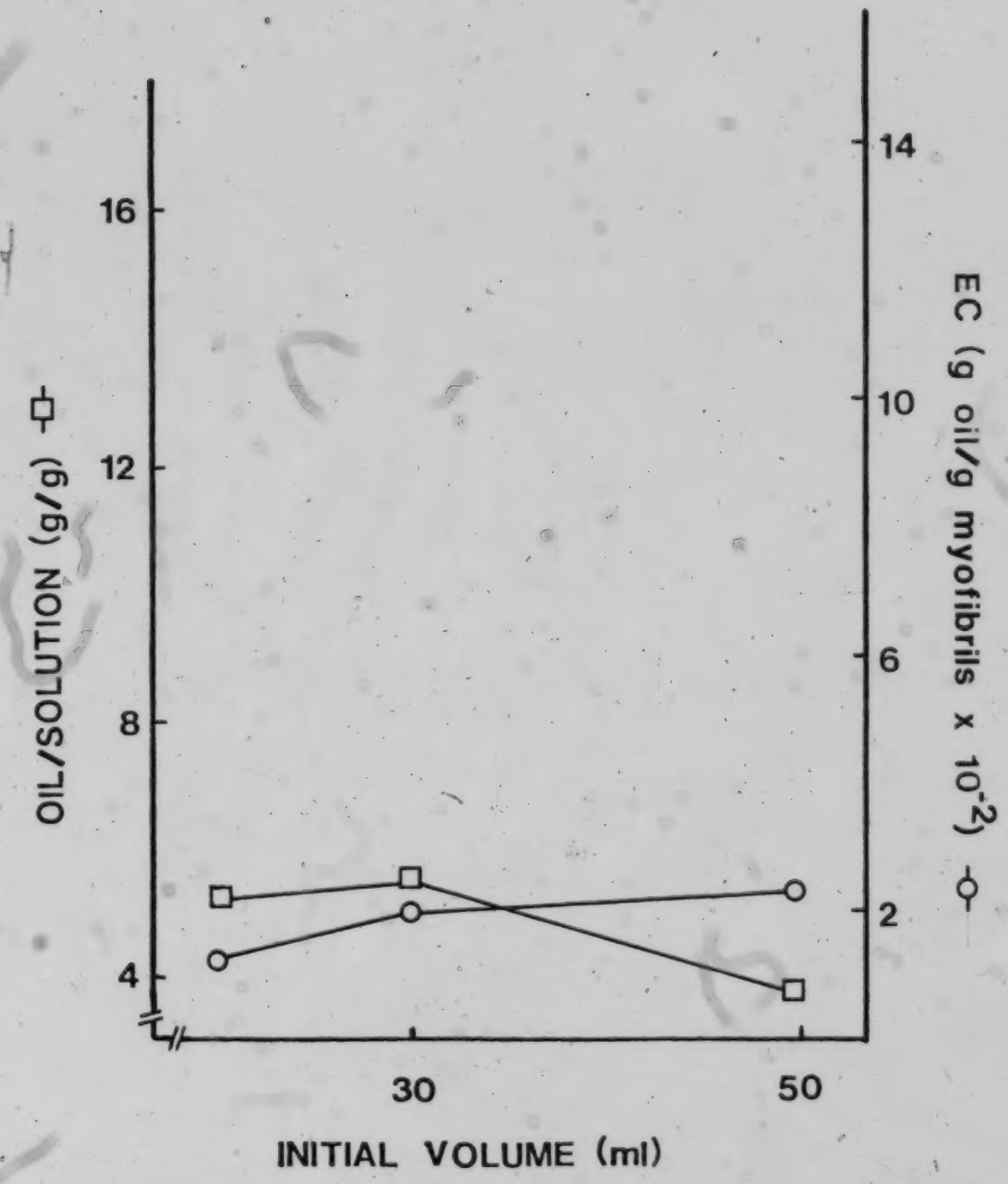

Figure 24: Effect of initial volume of solution on EC and the amount of oil emulsified for a myofibril solution by Method II

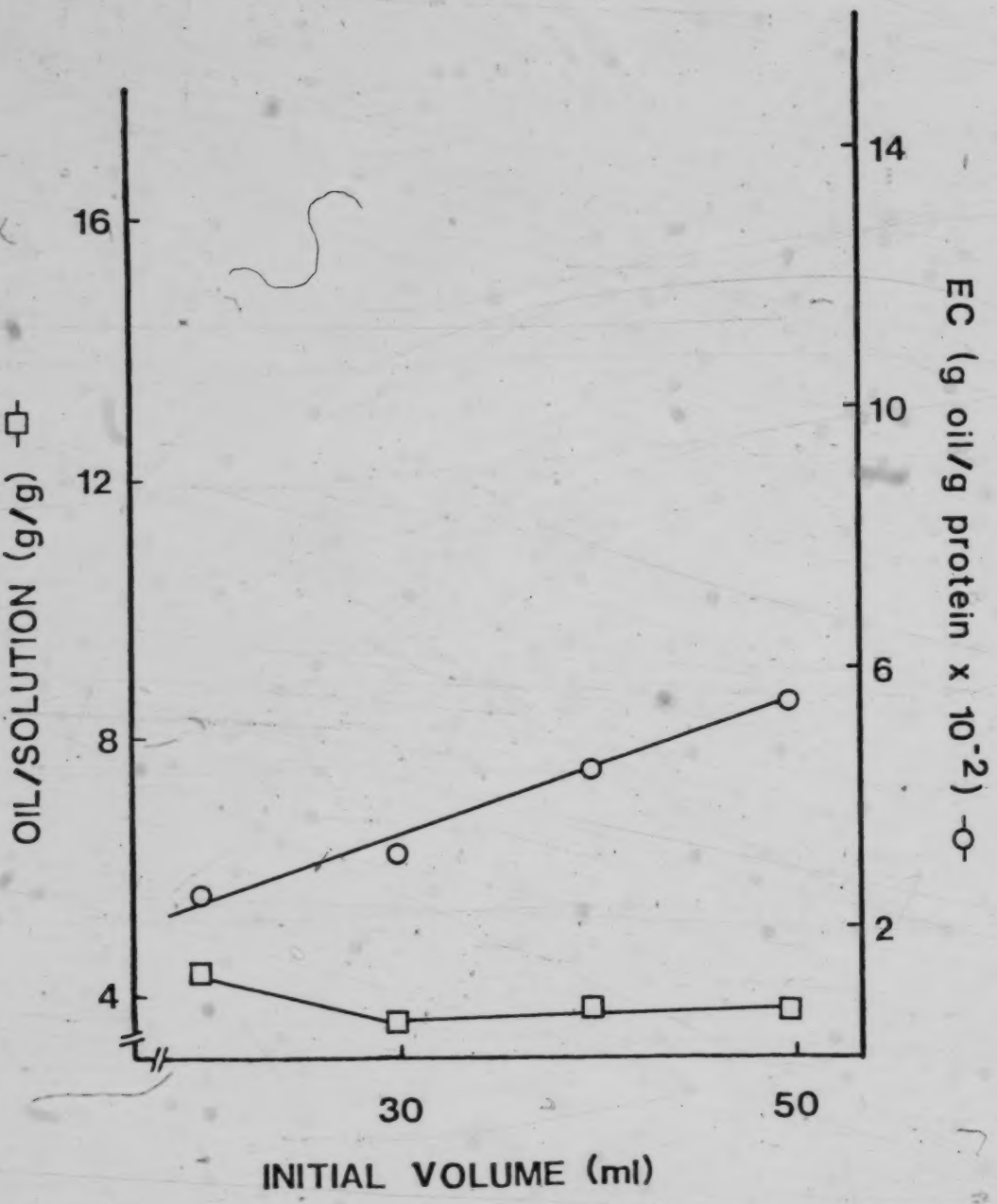

Figure 25: Effect of initial volume of solution on EC and the amount of oil emulsified for a cardiac muscle slurry by Method II

TABLE 31. Effect of protein concentration on the EC of a myofibril solution by Method II

Myofibrils [1]	Oil/solution	Average oil/solution	EC	Average EC
g	g/g	g/g	g oil/g myofibrils	g oil/g myofibrils
0	6,92 5,56	6,24	-- --	--
1	3,49 3,31	3,40	349,65 331,32	340,49
2	3,34 3,28	3,31	167,88 163,72	165,80
4	3,54 3,86	3,70	88,72 96,66	92,69
6	3,87 5,81	4,84	64,60 96,73	80,67
8	6,09 6,42	6,26	76,19 80,25	78,22

[1] g of myofibrils in 100 ml of solution

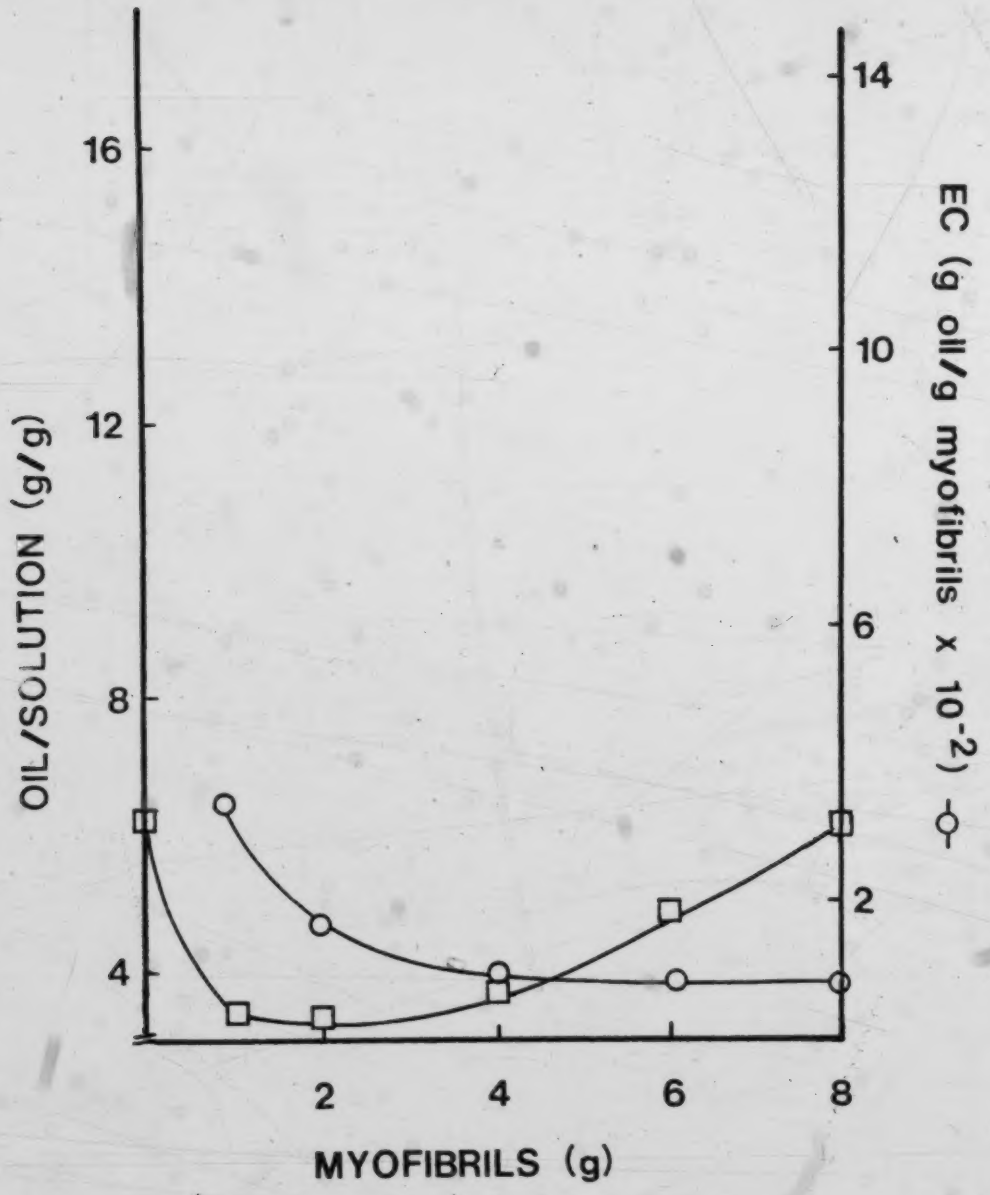

Figure 26: Effect of protein concentration on EC and the amount of oil emulsified for a myofibril solution by Method II

oil added increased with increasing concentration (figure 22). On the other hand, with salt alone there was no definite effect of concentration but the amount of oil added was relatively high although lower than in the presence of tris. No real emulsion could be formed with salt but the initial solution still had a low resistance while the solution containing a certain amount of oil offered infinite resistance to the passage of the current.

Problems associated with Method II

Using a propeller and trying to reproduce the experiments carried out by Borton *et al.* (1968), the problem of incomplete mixing near the end-point was encountered due to high viscosity of the emulsions and large amounts of oil. Accumulation of oil was unacceptable because of its influence on the end-point determination. Evidence that mixing near the end-point was of importance was provided when the addition of oil was stopped as the end-point was approached while the mixing was continued sometimes the emulsion collapsed without further addition of oil. After numerous trials, the problem of incomplete mixing was solved by stacking three identical propellers on top of each other on the same shaft. This system provided good mixing even when large amounts of oil were required but was not really convenient, because depending on whether the breakdown occurred at or between the propellers, the mixing action was different.

Especially when the rate of oil addition was considered, attempts to define standard conditions produced erratic results which were thought to be the result of variable speed of mixing, due to large variations in tachometer readings. This was later confirmed by an experiment on the

effect of the speed of mixing (table 29). It is thus difficult to compare values obtained at different times since the speed was adjusted frequently. Even when no adjustment was made i.e. even when the speed was not changed, variation in the speed occurred from one sample to the other due to the viscosity of the emulsion and to the poor quality of the mixer.

In spite of the variation in the speed of mixing, large amounts of oil were required to titrate a blank sample of tris buffer and this effect increased with increasing concentration of buffer (table 26, figure 22). Since salt concentration was found to have no effect on the amount of oil added (tables 24, 25), the effect of the buffer was attributed to tris. This effect of tris was reduced in the presence of myofibrils (table 31, figure 26) suggesting that the two phenomena are different. As in the case of the first method, it was clear that the EC, of the myofibrils in this case, decreased as the protein concentration was increased (table 31, figure 26). Since the speed of mixing was found to be an important factor and since it could not be controlled adequately using the Lightnin stirrer, Method III was designed to solve this particular problem.

Method III

The Fisher Stedi Speed stirrer is equipped with a control unit which adjusts the power supplied to the motor according to the viscosity of the solution so that the speed of mixing remains constant. Using this mixer, standard conditions were first determined using a standard protein (BSA) after which the EC of meat slurries and meat protein fractions was determined.

Effect of rate of oil addition

The effect of rate of oil addition was investigated using a 0,02% BSA solution in 1,0 M NaCl. The results presented in table 32 and figure 27 show that the amount of oil emulsified increased with increasing rates of oil addition. This is in good agreement with the data from Swift *et al.* (1961) (figure 7, from Swift *et al.* 1961). A rate of 20 ml/min was chosen for subsequent experiments because it constituted a good compromise between amount of oil and time required for the titration.

Effect of initial volume of solution

The effect of the initial volume of solution was determined as in the case of myofibrils in Method II. Twenty ml of 0,02% BSA solution were added to a 600 ml beaker to which increasing amounts of 1,0 M NaCl solution were added to give increasing initial volumes of protein solution containing the same amount of protein. Results showed that as the initial volume of protein solution was increased the amount of oil added during titration also increased (table 33, figure 28). A similar effect of the initial volume of solution was also reported by Carpenter and Saffle (1964) (figure 5, from Carpenter and Saffle 1964). A standard volume of 20 ml was used because it reduced the amount of oil required for the titration.

TABLE 32. Effect of rate of oil addition on the amount of oil emulsified by a 0,02% BSA solution by Method III

Rate	Oil/solution	Average oil/solution
ml/min	g/g	g/g
5	3,95 4,06 4,67	4,23
10	4,88 4,25 4,80	4,64
20	6,16 6,16 6,36	6,23
26	7,17 6,57 7,04	6,93
40	10,06 9,10 9,61	9,59
46	8,43 10,37 11,20	10,00

Effect of initial volume of oil

The effect of initial volume of oil added in the mixer before the titration itself was started was studied by Carpenter and Saffle (1964) who reported that the EC increased linearly with the initial volume of oil (table 3, from Carpenter and Saffle 1964); they attributed the effect of the initial volume of oil in part to differences in temperature resulting from different times of mixing. This observation could not be confirmed by the present experiment. In agreement with

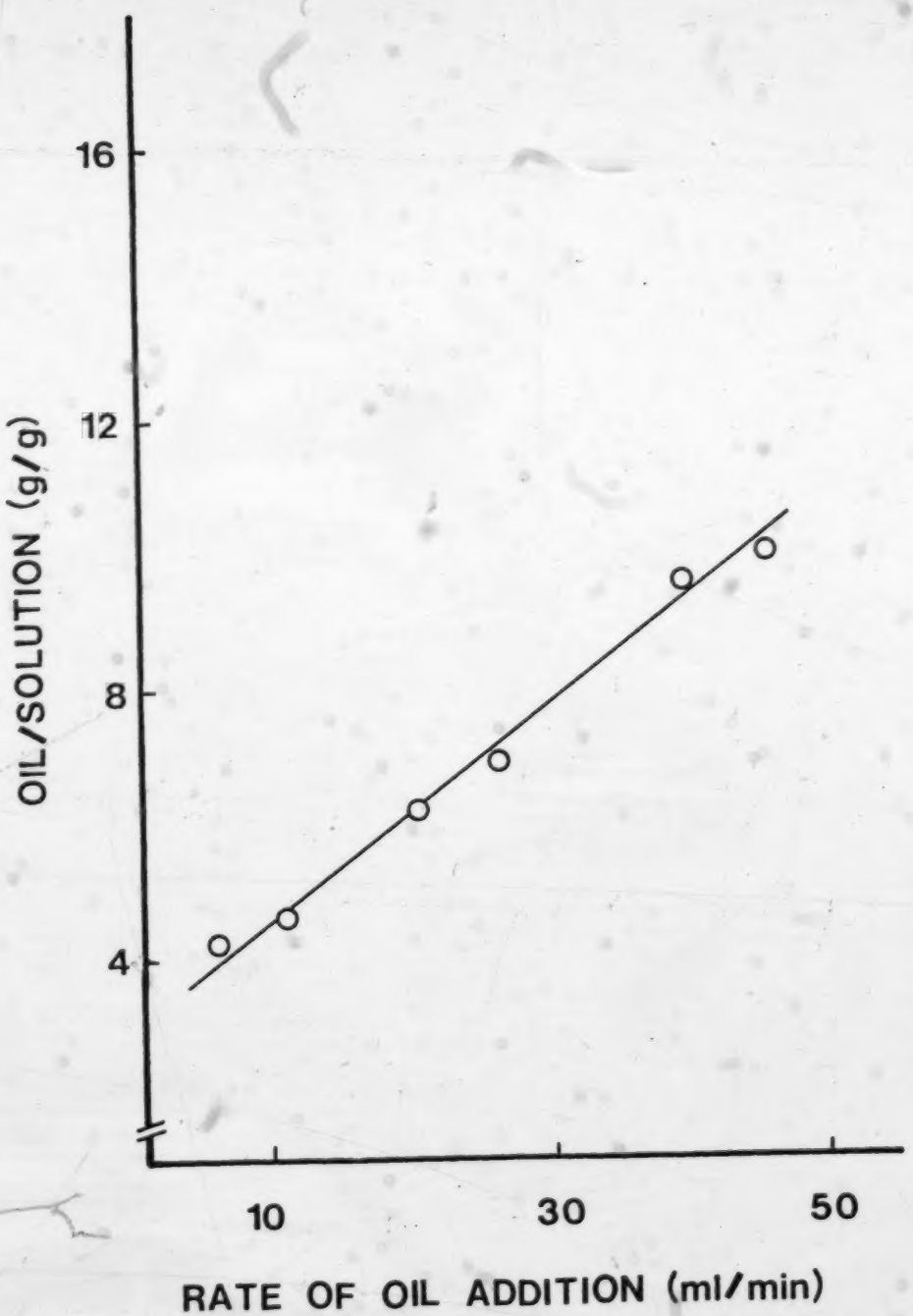

Figure 27: Effect of rate of oil addition on the amount of oil emulsified by a 0,02% BSA solution using Method III

TABLE 33. Effect of initial volume of solution on the amount on oil emulsified by a 0,02% BSA solution by Method III

Initial volume	Oil/solution	Average oil/solution
ml	g/g	g/g
20	6,58 5,96 6,18	6,24
30	7,71 8,17 4,85 [1]	7,94
40	10,34 9,40 10,06	9,93
50	12,11 11,33 14,50	12,65

[1] Discarded value

the results of the first experiment to study the effect of oil addition, more oil was emulsified at higher rate of addition. At low rate of oil addition (5 ml/min), no effect of the initial volume of oil could be found. Even at a higher rate of oil addition (20 ml/min), the initial volume of oil did not have much of an effect on the amount of oil emulsified even though the variation was larger (table 34). Since the initial amount of oil had no effect, it was decided not to add any oil before the titration was started for the subsequent experiments.

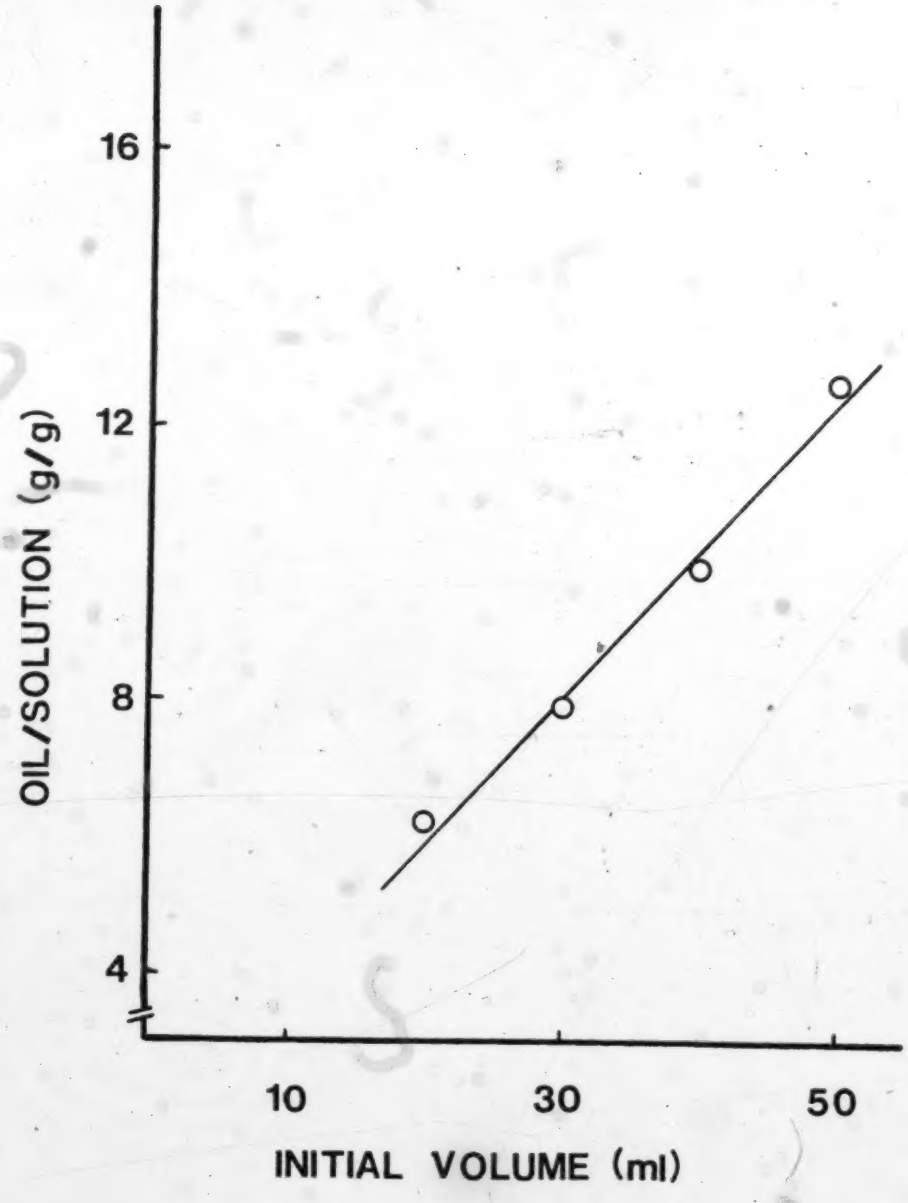

Figure 28: Effect of initial volume of solution on the amount of oil emulsified by a 0,02% BSA solution by Method III

TABLE 34. Effect of initial volume of oil on the amount of oil emulsified by a 0,02% BSA solution by Method III

Initial volume	20 ml of oil/min		5 ml of oil/min	
	Oil/solution	Average oil/solution	Oil/solution	Average oil/solution
ml	g/g	g/g	g/g	g/g
--	5,90 5,65 6,49	6,01	3,37 3,03 2,88	3,09
5	8,38 6,47 6,66	7,17	2,96 3,41 3,23	3,20
10	6,31 6,68 6,36	6,45	2,47 3,46 4,14	3,36
20	6,27 5,77 6,07	6,04	2,91 3,00 2,95	2,95
25	-- -- --	--	3,23 2,97 3,93	3,38

Effect of salt

In order to determine the effect of salt on the amount of oil added during titration, solutions containing an increasing concentration of salt were tested with and without protein. A 0,02% BSA solution was used in cases where the protein was present. Results of this experiment are reported in table 35. Curves obtained from these results show that salt concentration did not have any effect in the absence of protein. In the presence of protein the amount of oil emulsified had a tendency to slightly decrease as the salt concentration

TABLE 35. Effect of salt concentration on the amount of oil added to salt solutions with and without protein by Method III

Salt concentration	Oil/solution		Average oil/solution	
	g/g		g/g	
M	no protein	protein	no protein	protein
-	-- -- --	7,50 9,07 5,92	--	7,50
0,15	4,57 5,41 4,59	5,98 6,44 6,39	4,86	6,27
0,6	4,99 4,69 3,59	6,13 6,20 6,05	4,42	6,13
1,0	5,64 4,92 5,10	5,93 6,25 6,02	5,22	6,07
2,0	4,22 4,42 2,77 [1]	5,77 5,33 5,61	4,32	5,57

[1] Discarded value

was increased. It is expected that the effect of salt will be different for each protein. However, it should be noted that the amount of oil added was not greatly lowered by the removal of the protein. In subsequent experiments, the salt concentration was constant at 1,0 M unless a buffer had to be used.

Effect of pH

Similarly, the effect of pH on the amount of oil added was studied in the absence and in the presence of protein at least for certain pH values. Results are reported in table 36 and figure 29. It is difficult to say anything about the effect of pH in the absence of protein because of the limited number of data points, the effect reported in figure 29 could be due to large experimental error. For BSA, the amount of oil added was minimum at pH 7.5 but the variation was quite small. As for salt, the effect of pH is expected to be different for each protein. The pH of the meat slurries and protein solutions was adjusted to 6.0 while that of the muscle protein fractions was that of the buffer i.e. 7.6.

Effect of tripolyphosphate

To investigate the effect of tripolyphosphate on the amount of oil added, conditions prevailing in a 0,02% BSA solution were reproduced. The molar concentration of the more dilute tripolyphosphate solution was the same as that of the BSA solution and was similarly prepared with 1,0 M NaCl solution. At the same molar level of 0,03 mM, the amount of oil added was greater for the protein alone (table 37).

TABLE 36. Effect of pH on the amount of oil added to salt solutions with and without protein by Method III

pH	Oil/solution g/g		Average oil/solution g/g	
	no protein	protein [1]	no protein	protein [1]
5.0	4,30 4,42 3,02	7,33 6,99 6,47	3,91	6,56
5.5	-- -- --	6,98 7,55 6,99	--	7,17
5.75	-- -- --	6,37 6,26 7,05	--	6,56
6.0	2,71 4,73 3,00	7,31 6,87 7,24	3,48	7,14
6.5	-- -- --	6,05 6,17 5,93	--	6,05
7.0	5,30 4,82 5,38	5,56 5,40 5,39	5,17	5,45
7.5	-- -- --	4,77 5,18 5,07	--	5,01
8.0	-- -- --	6,71 6,07 5,44	--	6,07

[1] 0,02% BSA

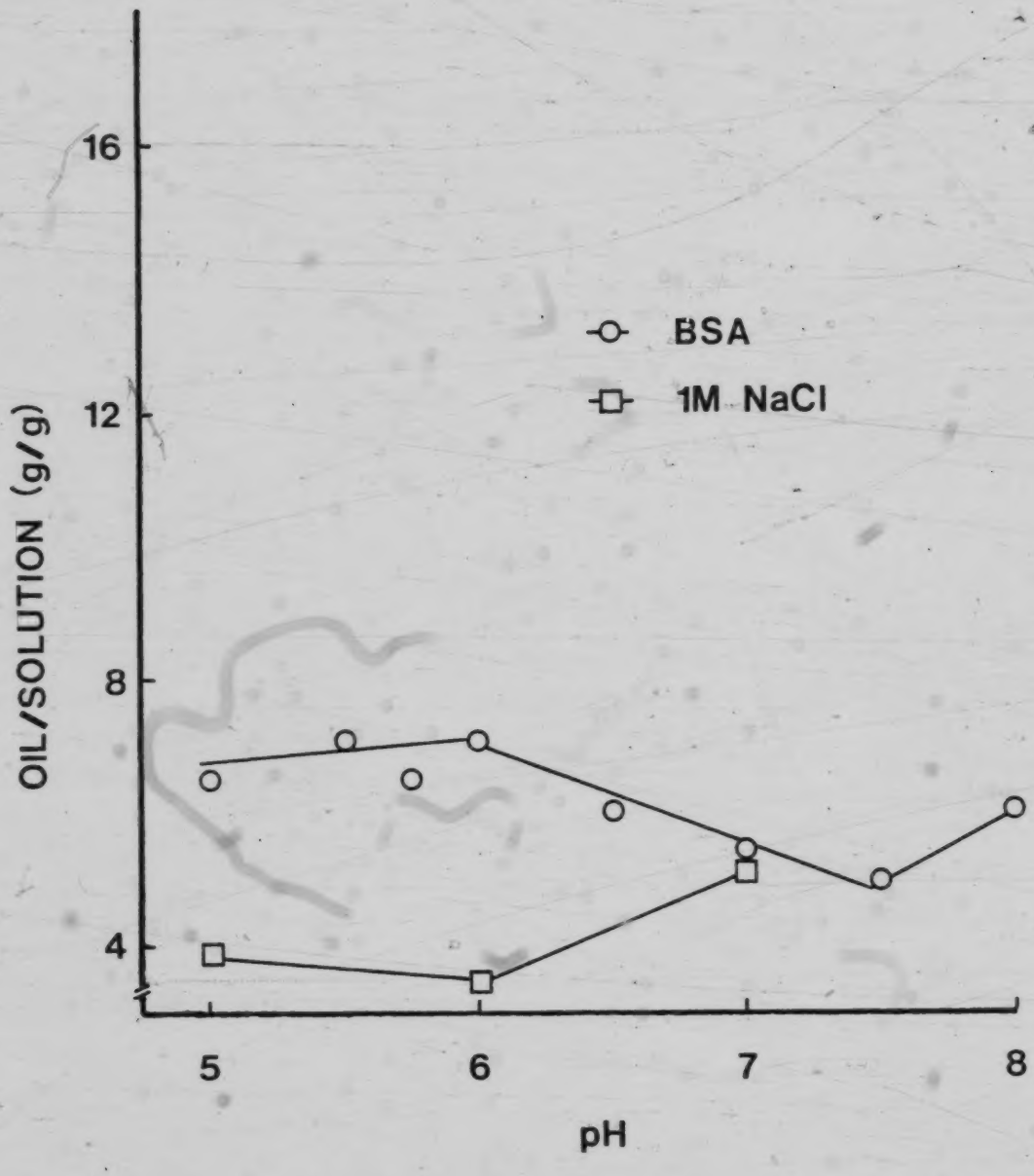

Figure 29: Effect of pH on the amount of oil added to salt solutions with and without protein by Method III

TABLE 37. Effect of tripolyphosphate concentration on the amount of oil added by Method III.

Concentration	Oil/solution	Average oil/solution
mM	g/g	g/g
0,03 (BSA)	5,90 6,00 6,83	6,24
0,03	4,14 4,32 4,44	4,30
0,3	5,03 4,49 4,17	4,56
3,0	5,34 5,01 5,14	5,16

With increasing concentrations of tripolyphosphate, only a slight increase in the amount of oil needed for titration was observed.

Effect of protein concentration

The effect of protein concentration was studied for BSA solutions, skeletal and cardiac muscle slurries and also for the sarcoplasmic and myofibrillar proteins extracted from those two muscles. The effect of protein concentration is again the same in all cases, the relationship being negative and most of the time curvilinear between EC and the protein concentration.

BSA solutions

BSA solutions containing from 0,1 to 0,3 mg protein/g solution were used to determine the effect of protein concentration. Values of EC and amounts of oil emulsified at different levels are presented in table 38 and reported on figure 30.

TABLE 38. Effect of protein concentration on EC by Method III (BSA)

Protein content	Oil/solution	Average oil/solution	EC	Average EC
mg/g	g/g	g/g	g oil/mg protein	
0,1	4,59 4,80 4,67	4,69	45,9 48,0 46,7	46,9
0,2	6,65 5,99 5,31	5,98	33,3 30,0 26,6	30,0
0,3	8,98 9,00 8,52	8,83	30,0 30,0 28,4	29,5

Skeletal and cardiac muscle slurries

The effect of protein concentration was studied for both skeletal and cardiac muscle slurries, before and after preblending at protein levels requiring reasonable amounts of oil. The results for skeletal muscle are presented in table 39 and those for cardiac muscle in table 40. It happened that the convenient protein levels did not correspond making it difficult to compare results as can be seen in figures 31 and 33 for the EC and figures 32 and 34 for the amount of oil emulsified.

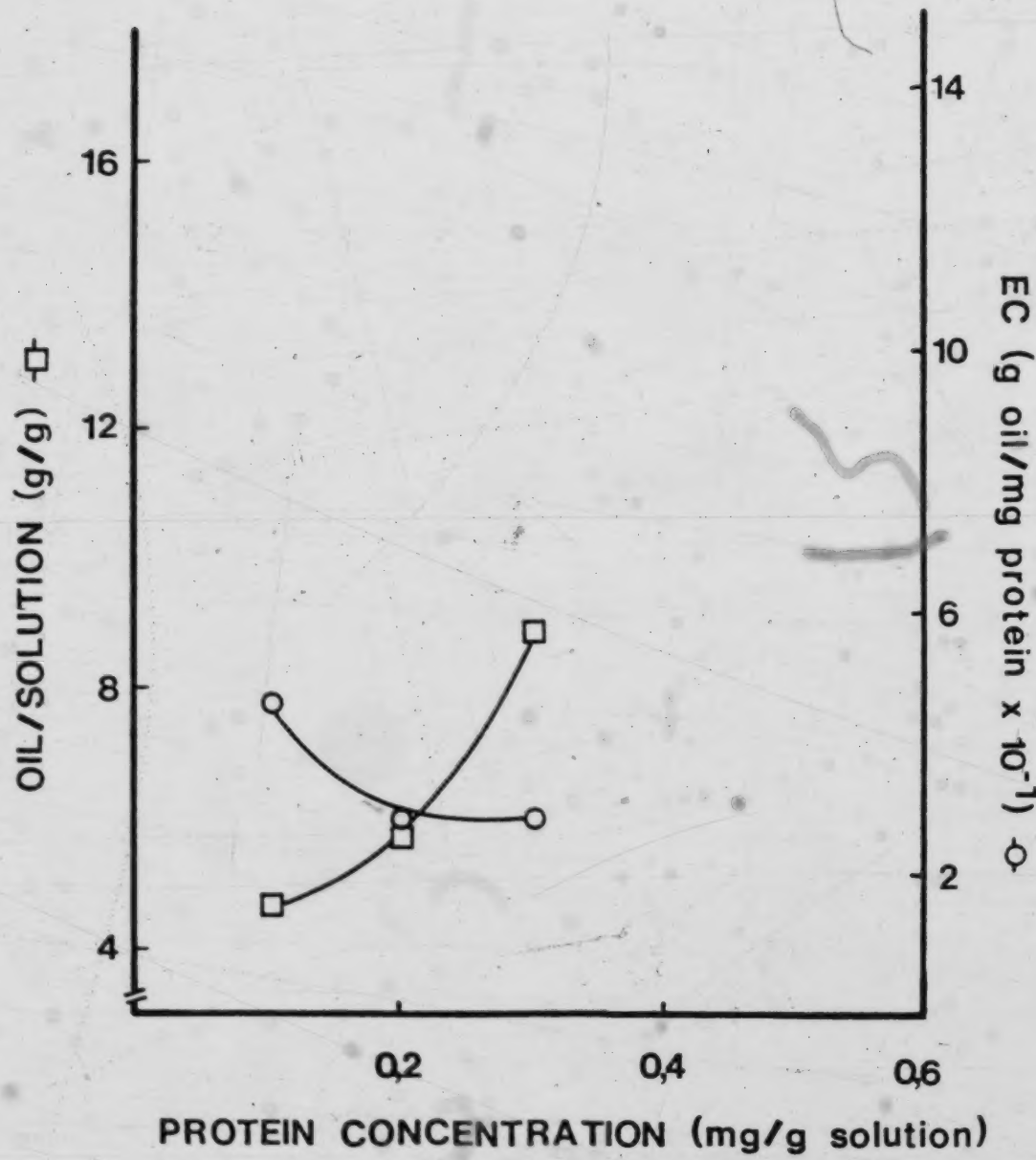

Figure 30: Effect of protein concentration on EC and the amount of oil emulsified for BSA solutions by Method III

TABLE 39. Effect of protein concentration on EC of skeletal muscle slurry by Method III

Protein concentration	Before preblending				After preblending			
	Oil/slurry	Average oil/slurry	EC	Average EC	Oil/slurry	Average oil/slurry	EC	Average EC
mg/g slurry	g/g	g/g	g oil/mg protein		g/g	g/g	g oil/mg protein	
2,0	6,28 7,26 6,46	6,67	2,85 3,30 2,94	3,03	8,21 7,21 6,56	7,33	3,73 3,28 2,98	3,33
2,5	7,50 7,64 7,93	7,69	2,78 2,83 2,94	2,85	7,03 7,47 7,55	7,35	2,60 2,77 2,80	2,72
5,0	9,25 9,26 9,05	9,19	1,85 1,85 1,81	1,84	8,48 9,04 8,25	8,59	1,70 1,81 1,65	1,72

TABLE 40. Effect of protein concentration on EC of cardiac muscle slurry by Method III

Protein concentration	Before preblending				After preblending			
	Oil/slurry	Average oil/slurry	EC	Average EC	Oil/slurry	Average oil/slurry	EC	Average EC
mg/g slurry	g/g	g/g	g oil/mg protein		g/g	g/g	g oil/mg protein	
0,5	5,28 5,36 5,99	5,54	10,56 10,72 11,98	11,09	5,88 5,77 5,64	5,76	11,76 11,54 11,28	11,53
1,5	9,68 9,57 9,75	9,67	6,45 6,38 6,50	6,44	10,22 9,90 10,71	10,28	6,81 6,60 7,14	6,85
2,0	10,79 10,54 10,72	10,68	5,40 5,27 5,36	5,34	10,96 10,44 13,14	10,68	5,48 5,22 6,57	5,76

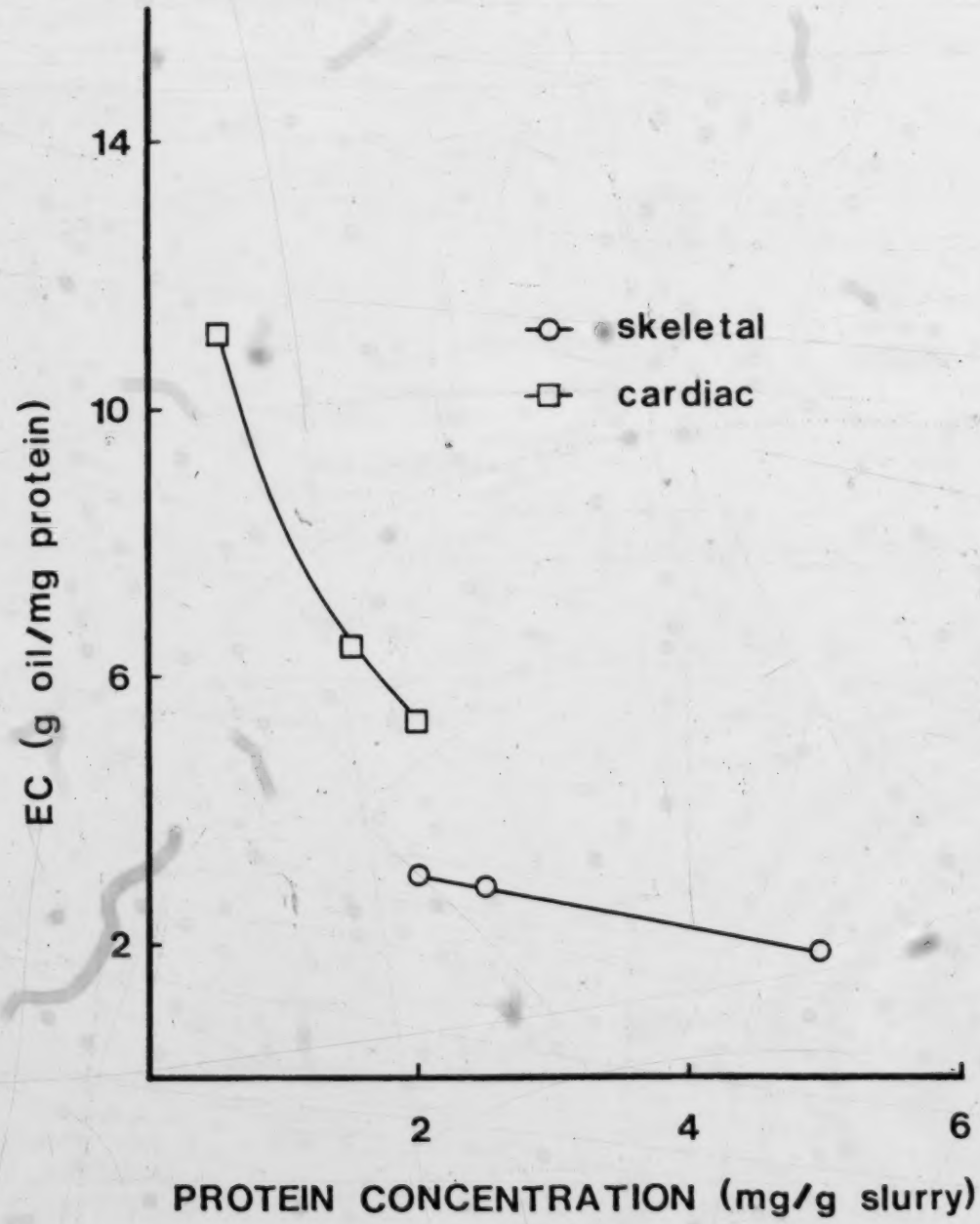

Figure 31: Effect of protein concentration on EC of skeletal and cardiac muscle slurries before preblending by Method III

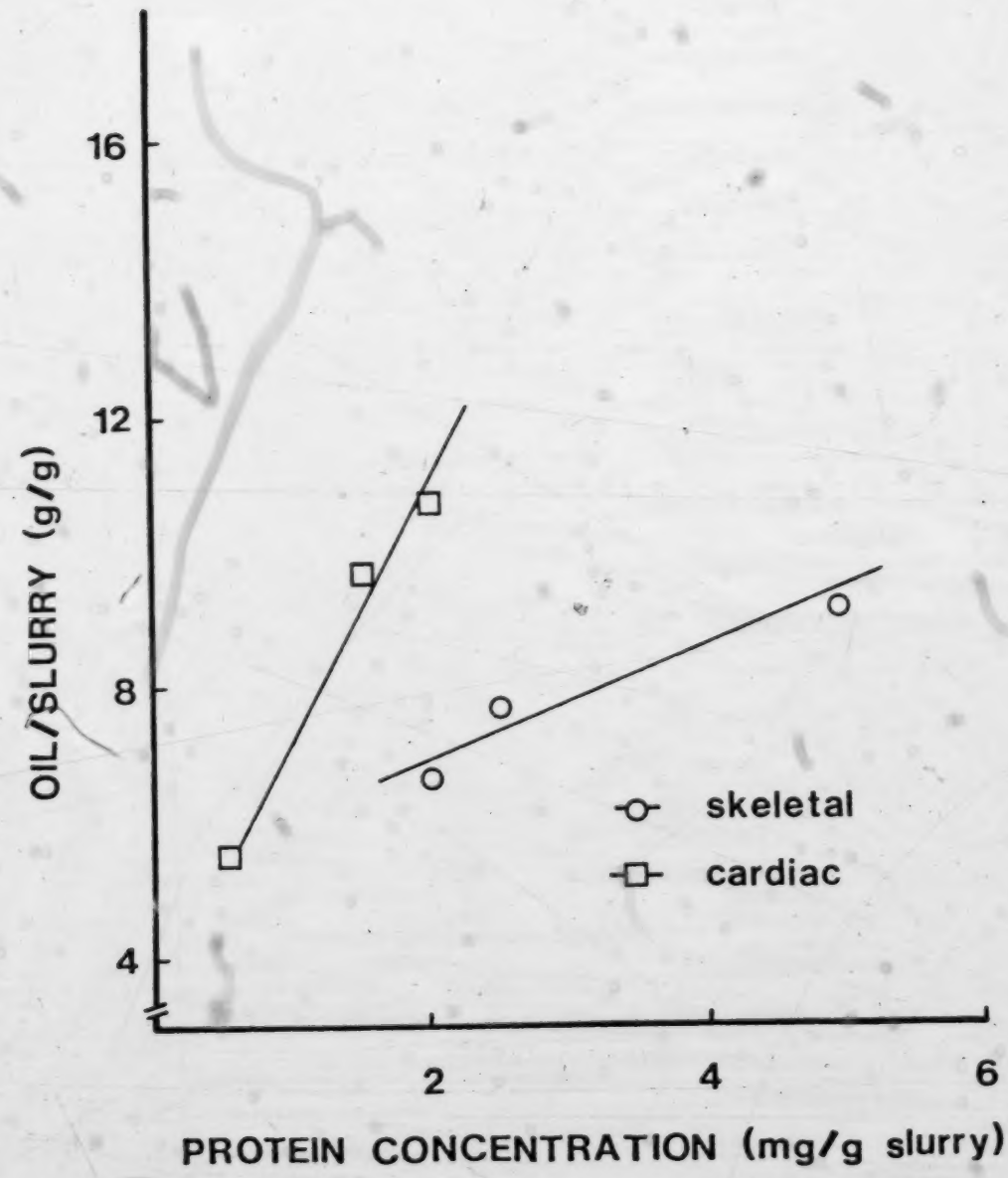

Figure 32: Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries before preblending by Method III

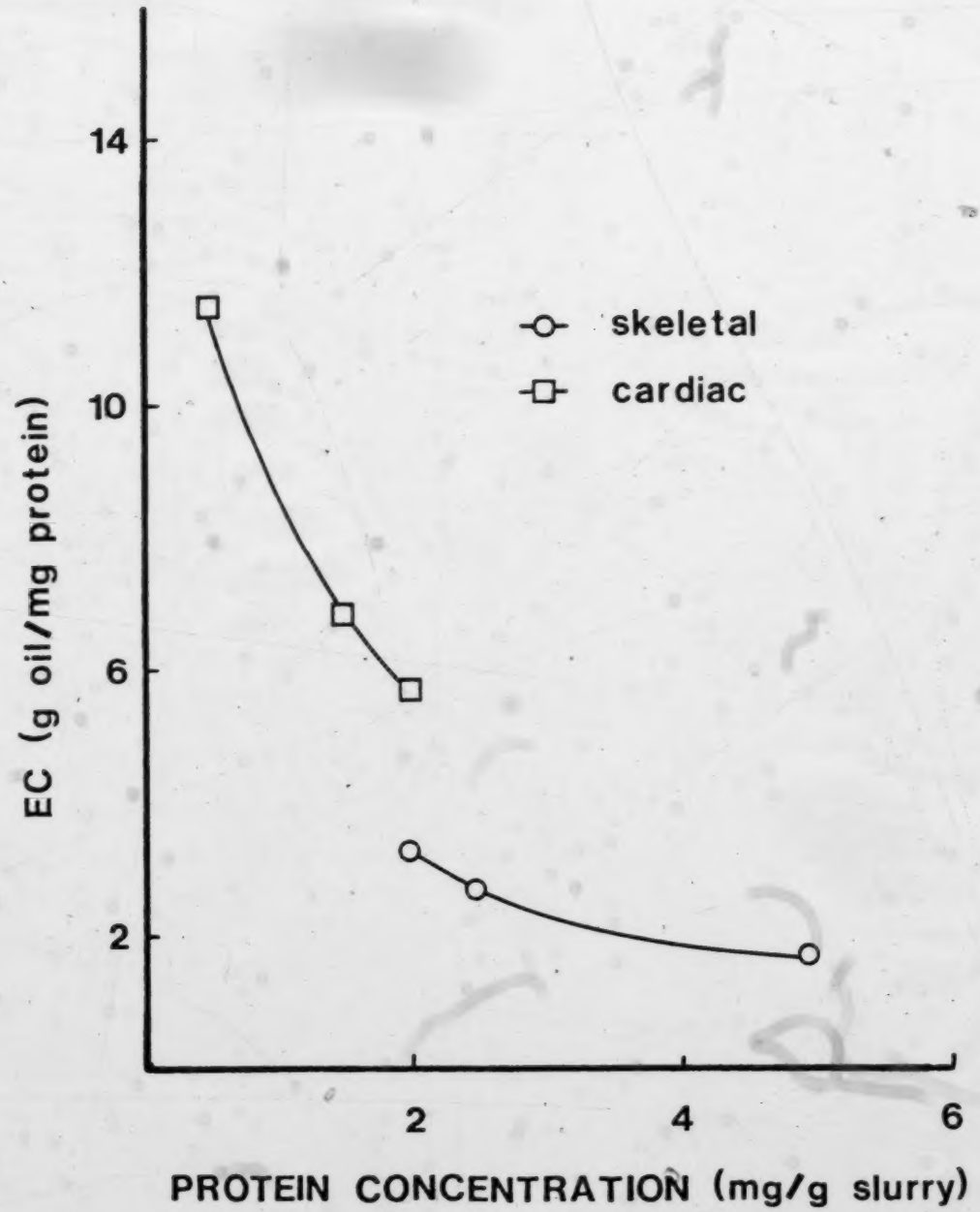

Figure 33: Effect of protein concentration on EC of skeletal and cardiac muscle slurries after preblending by Method III

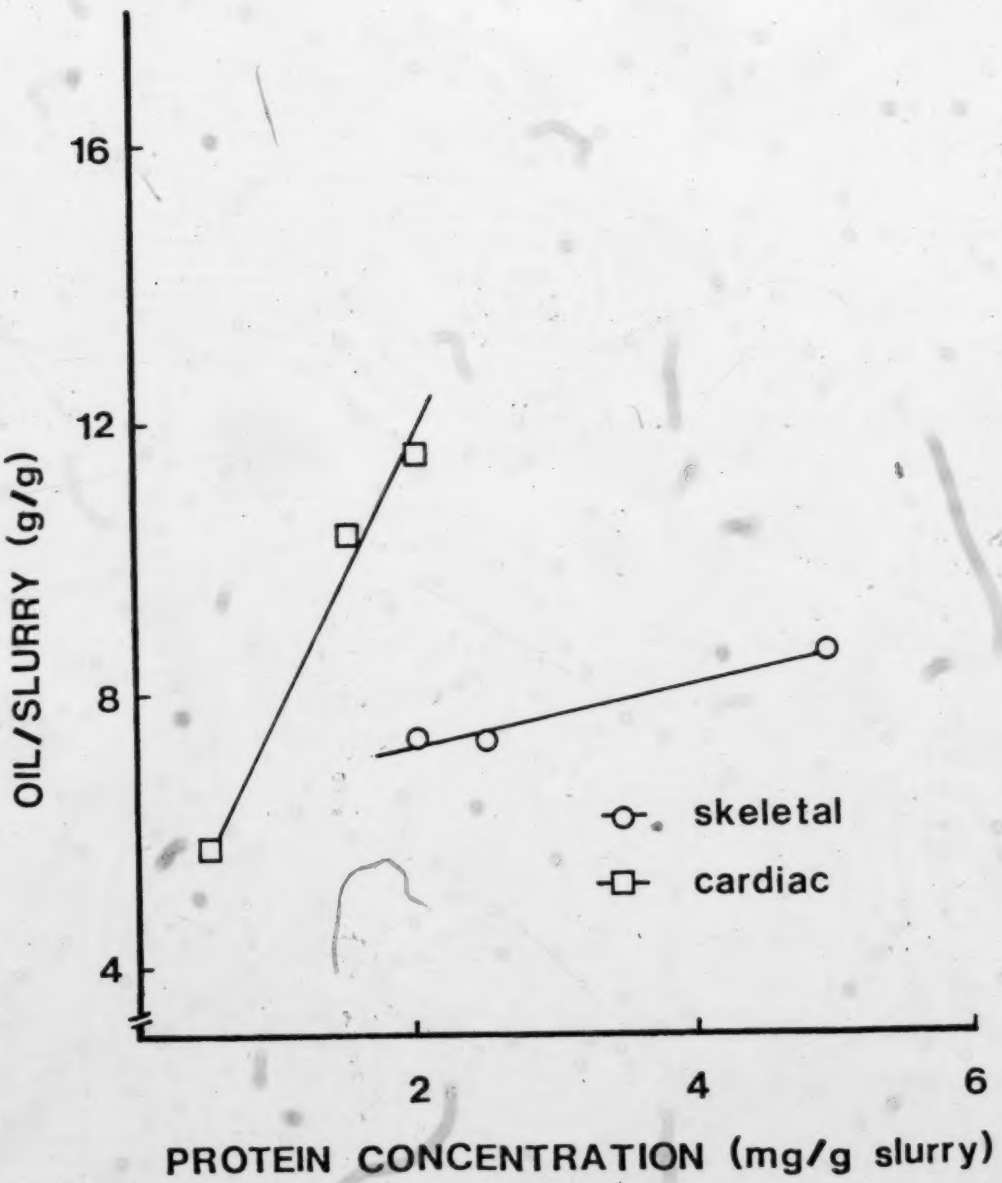

Figure 34: Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries after preblending by Method III

According to figures 31 and 33 for EC it seems that the EC of cardiac muscle slurries is superior to that of skeletal muscle slurries. As far as the amount of oil emulsified is concerned, the same comparison is valid, cardiac muscle being superior to skeletal muscle. But in order to make a valid comparison, similar proteim levels should be employed. Therefore, the effect of protein concentration was determined again at comparable protein levels for skeletal and cardiac muscle slurries. These results are presented in tables 41 amd 42 for skeletal and cardiac muscle slurries respectively. The corresponding curves of EC appearing in figure 35 clearly show that although the difference is small, the EC of cardiac muscle slurries is superior to that of skeletal muscle slurries. As usual, the EC decreases as the protein concentration is increased. The amount of oil emulsified is slightly superior for cardiac muscle slurries and increases as the protein concentration is increased (figure 36).

When numerical values for meat slurries and BSA solutions are compared, it can be seen that much lower levels of BSA were needed in order to get reasonable amounts of oil emulsified. Since the amounts of oil emulsified by these dilute BSA solutions are comparable to those emulsified by muscle slurries, the EC values for the BSA solutions are considerably higher.

Sarcoplasmic and myofibrillar proteins

Sarcoplasmic and myofibrillar proteins were extracted from both skeletal and cardiac muscles and the EC of the resulting protein solutions was determined at comparable protein levels. Results for

TABLE 41. Effect of protein concentration on EC of skeletal muscle slurries by Method III

Protein concentration	Oil/slurry	Average oil/slurry	EC	Average EC
mg/g slurry	g/g	g/g	g oil/mg protein	
0,5	5,31 5,55 5,02	5,29	10,62 11,10 10,04	10,59
1,0	6,25 7,39 7,62	7,09	6,25 7,39 7,62	7,09
1,99	10,38 9,81 10,12	10,10	5,22 4,93 5,09	5,08

TABLE 42. Effect of protein concentration on EC of cardiac muscle slurries by Method III

Protein concentration	Oil/slurry	Average oil/slurry	EC	Average EC
mg/g slurry	g/g	g/g	g oil/mg protein	
0,49	6,84 6,01 6,51	6,45	13,96 12,27 13,29	13,17
0,98	9,08 8,69 9,55	9,11	9,27 8,87 9,74	9,29
1,97	12,12 11,58 11,94	11,88	6,15 5,88 6,06	6,03

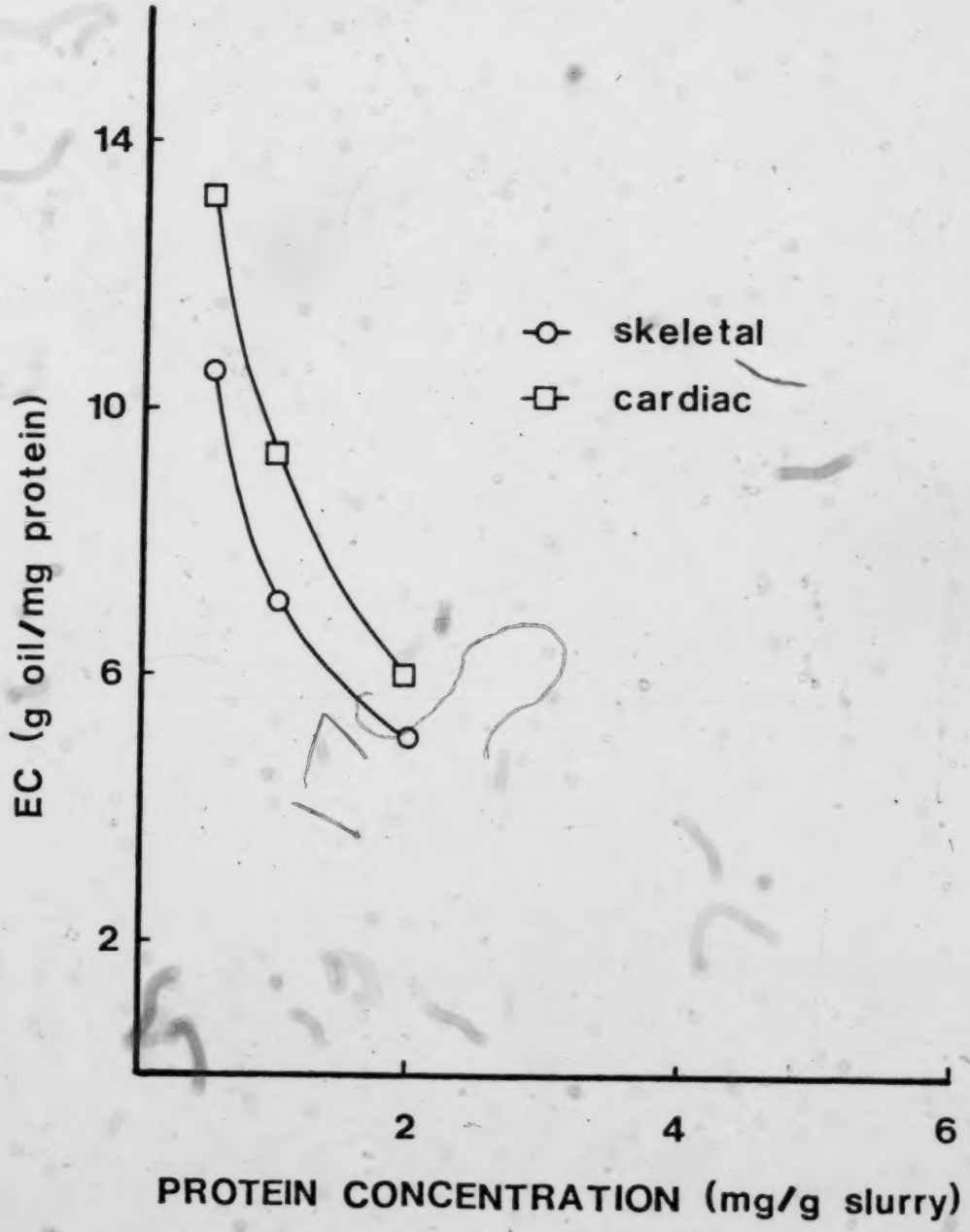

Figure 35: Effect of protein concentration on EC of skeletal and cardiac muscle slurries before preblending by Method III

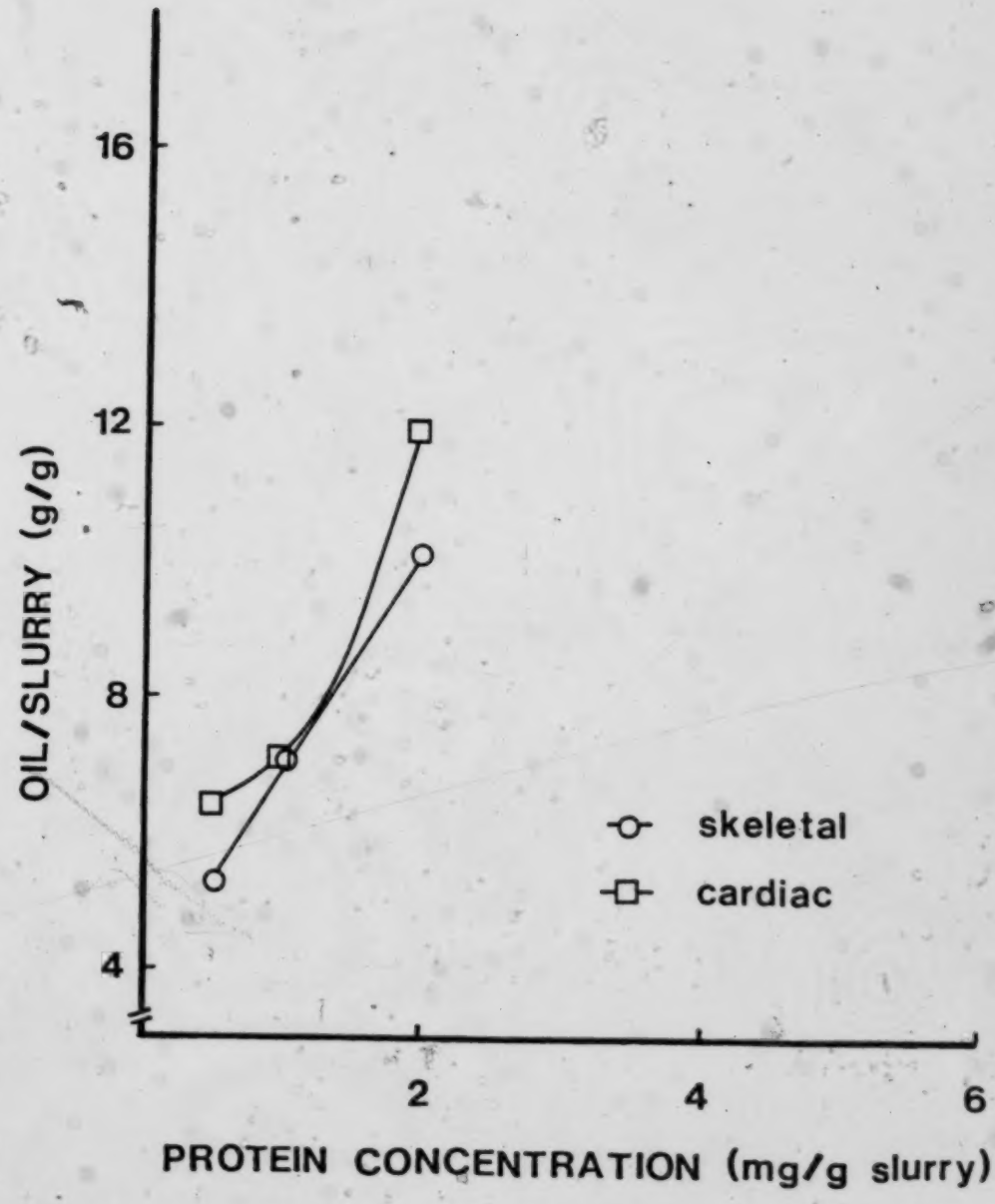

Figure 36: Effect of protein concentration on the amount of oil emulsified by skeletal and cardiac muscle slurries before preblending by Method III

sarcoplasmic proteins are presented in table 43 for skeletal muscle and table 44 for cardiac muscle. The EC of the sarcoplasmic proteins from the cardiac muscle is slightly higher than that of the sarcoplasmic proteins from the skeletal muscle and so was the amount of oil emulsified since the protein levels are the same in both cases. This can easily be seen from figures 37 and 38. Results for myofibrillar proteins appear in tables 45 and 46 for skeletal and cardiac muscles respectively. Curves of EC and the amount of oil emulsified obtained from these results are presented in figures 39 and 40. Conclusions similar to those obtained for the sarcoplasmic proteins can be drawn for the myofibrillar proteins. When myofibrillar proteins are compared to sarcoplasmic proteins for the same muscle, the EC of myofibrillar proteins is lower than that of sarcoplasmic proteins for both skeletal and cardiac muscles.

From the study of the effect of protein concentration, it can be concluded that the EC of all proteins investigated decreases as the protein concentration is increased while the amount of oil emulsified by these protein solutions increases with protein concentration. It also becomes apparent that the cardiac muscle is slightly superior to the skeletal muscle and that is true for muscle slurries as well as for the muscle protein fractions. Sarcoplasmic proteins were found to be more efficient than myofibrillar proteins.

Effect of preblending

The effect of preblending was presented in the previous study on the effect of protein concentration in tables 39 and 40. Standing in a

TABLE 43. Effect of protein concentration on EC of sarcoplasmic proteins from skeletal muscle by Method III

Protein concentration	Oil/solution	Average oil/solution	EC	Average EC
mg/g solution	g/g	g/g	g oil/mg protein	
1,0	7,66 7,82 5,86	7,11	7,66 7,82 5,86	7,11
2,0	8,74 9,25 8,97	8,99	4,37 4,63 4,49	4,50
2,5	9,81 10,07 10,33	10,07	3,92 4,03 4,13	4,03
5,0	15,20 15,78 15,56	15,51	3,04 3,16 3,11	5,10

TABLE 44. Effect of protein concentration on EC of sarcoplasmic proteins from cardiac muscle by Method III

Protein concentration	Oil/solution	Average oil/solution	EC	Average EC
mg/g solution	g/g	g/g	g oil/mg protein	
1,0	8,19 8,33 8,53	8,35	8,19 8,33 8,53	8,35
2,0	10,82 10,29 10,54	10,55	5,41 5,14 5,27	5,27
2,5	11,45 11,74 11,08	11,42	4,58 4,70 4,43	4,57

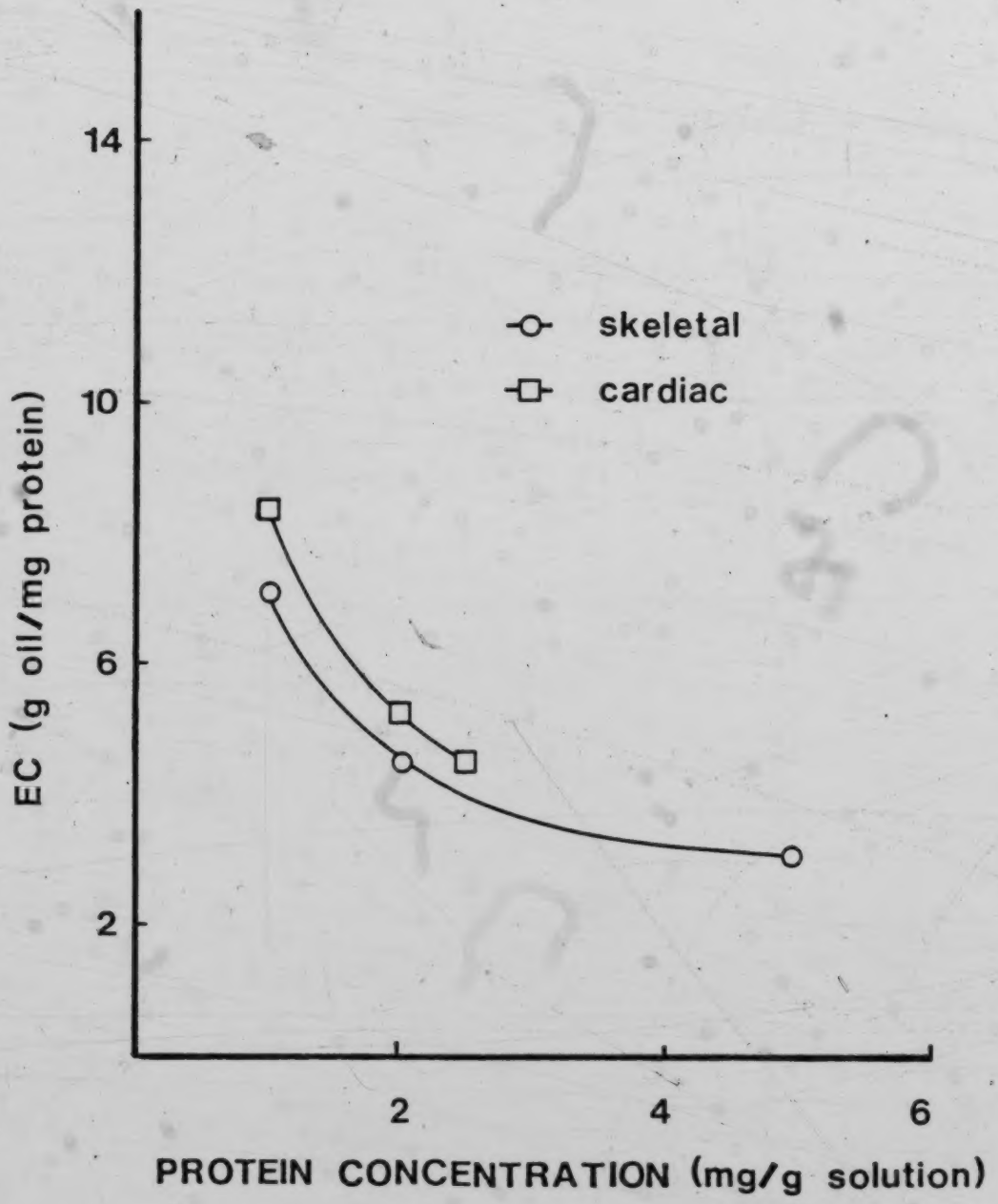

Figure 37: Effect of protein concentration on EC of sarcoplasmic proteins by Method III

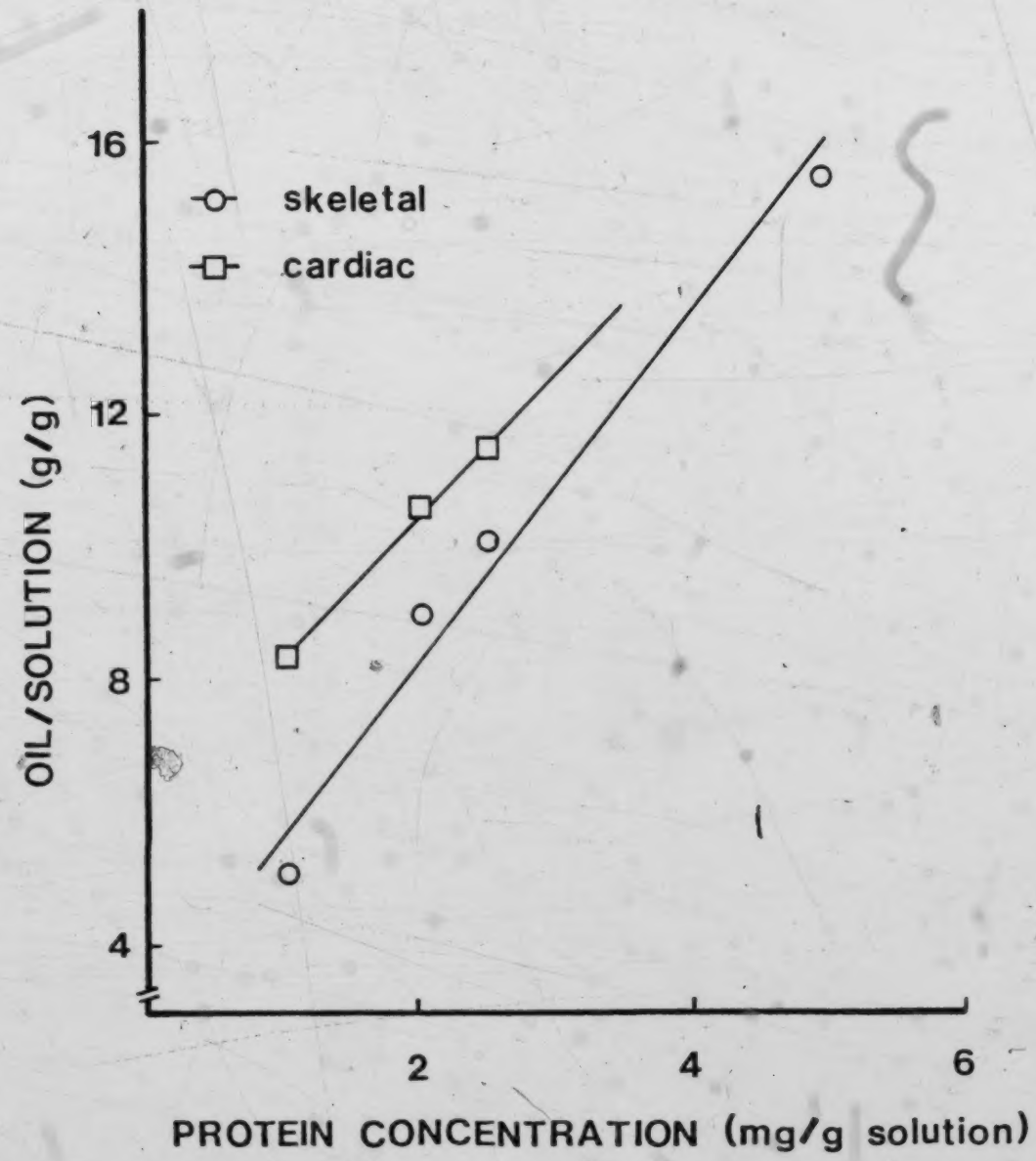

Figure 38: Effect of protein concentration on the amount of oil emulsified by sarcoplasmic proteins by Method III

TABLE 45. Effect of protein concentration on EC of myofibrillar proteins from skeletal muscle by Method III

Protein concentration	Oil/solution	Average oil/solution	EC	Average EC
mg/g solution	g/g	g/g	g oil/mg protein	
2,0	3,69 4,18 2,99	3,62	1,85 2,09 1,50	1,81
2,5	4,02 3,99 3,73	3,91	1,61 1,60 1,49	1,57
5,0	4,33 4,88 5,20	4,80	0,87 0,98 1,04	0,96

TABLE 46. Effect of protein concentration on EC of myofibrillar proteins from cardiac muscle by Method III

Protein concentration	Oil/solution	Average oil/solution	EC	Average EC
mg/g solution	g/g	g/g	g oil/mg protein	
1,0	3,75 4,55 4,23	4,18	3,75 4,55 4,23	4,18
2,5	7,11 6,42 5,99	6,51	2,84 2,59 2,40	2,60
5,0	8,70 8,68 10,31	9,23	1,74 1,74 2,06	1,85

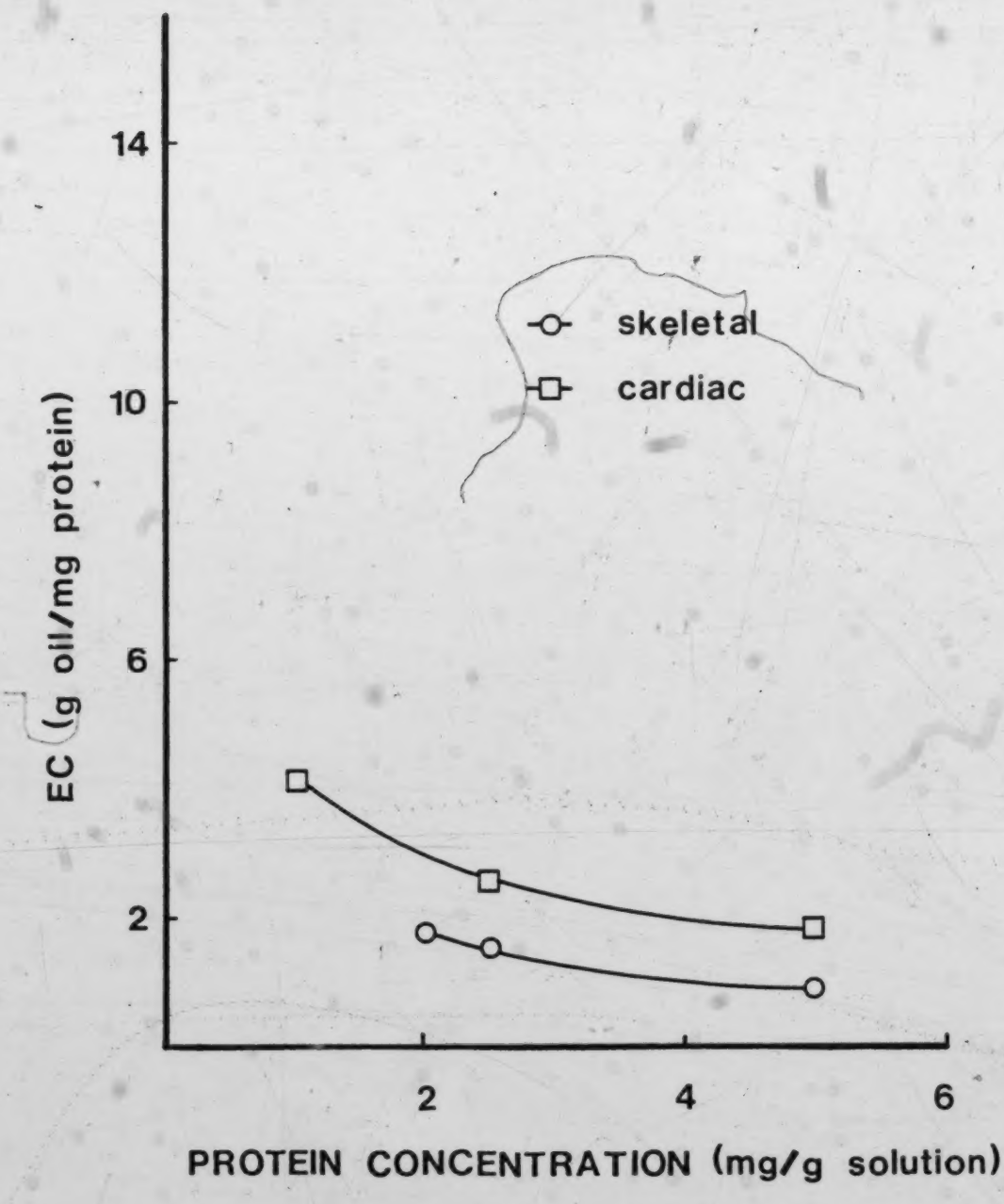

Figure 39: Effect of protein concentration on EC of myofibrillar proteins by Method III

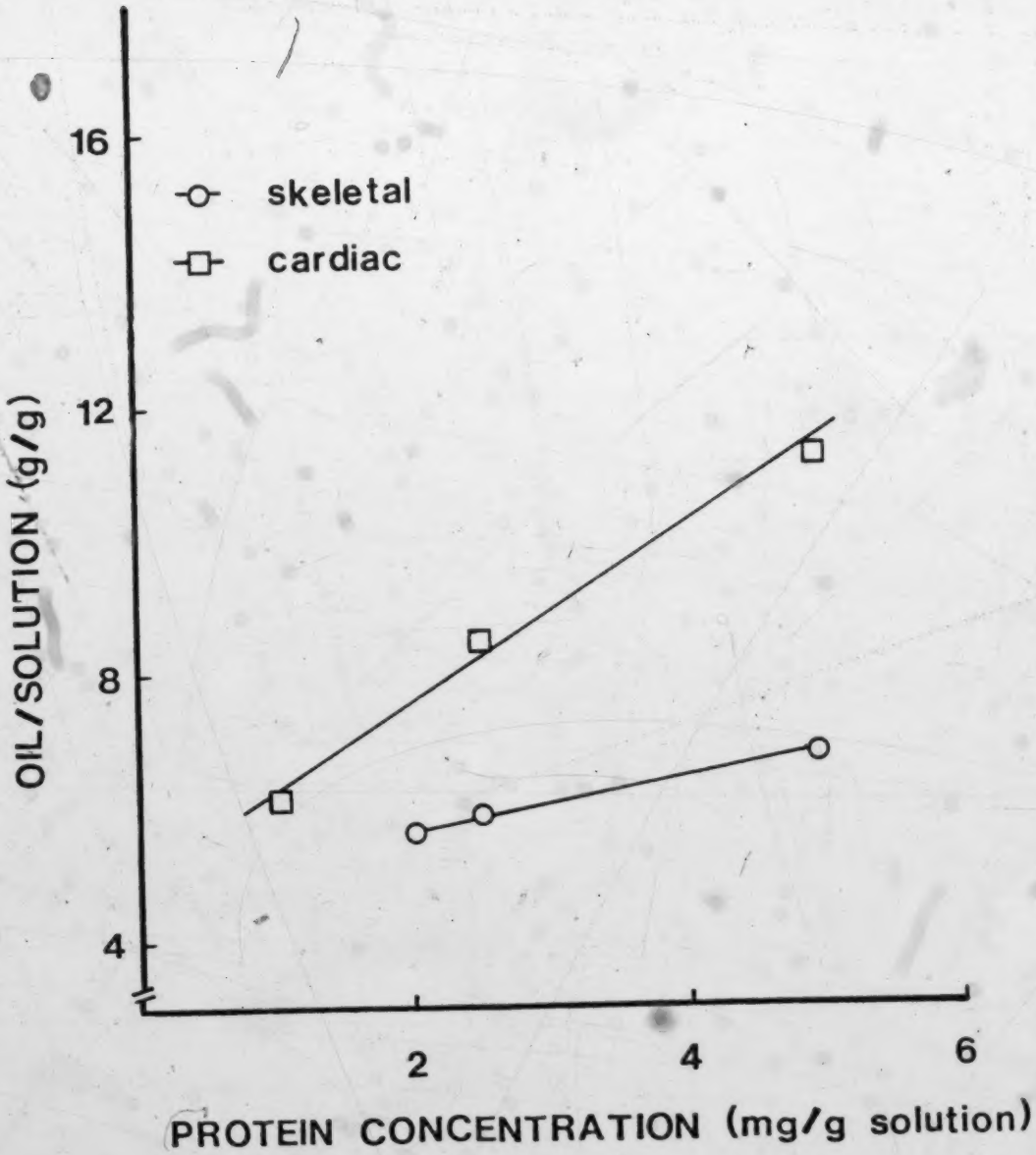

Figure 40: Effect of protein concentration on the amount of oil emulsified by myofibrillar proteins by Method III

salt solution at 2°C for 24 hours to extract the salt-soluble proteins had no effect since the EC value for both skeletal and cardiac muscle slurries after such a treatment was the same as for the freshly prepared slurries.

Effect of enzymatic digestion

Enzymatic digestion of skeletal and cardiac muscle slurries was followed by measuring the viscosity of the slurries and its effect on EC was determined after different periods of time. The viscosity of the skeletal muscle slurry decreased gradually with time of exposure to the enzyme action indicating that the enzyme was active (table 47, figure 41). The EC of the skeletal muscle slurry also decreased with time during the first 24 hours after which it started to increase again (table 47, figure 42). While the much lower viscosity of the cardiac muscle slurry also decreased to a lesser extent, there was no trend in EC (table 48, figure 42). From the previous results, it is difficult to establish a relationship between enzymatic digestion as followed by the viscosity and EC. As the proteolysis proceeds and the proteins are broken down, the EC should decrease which is the case here for the skeletal muscle slurry. However, after prolonged hydrolysis, the EC almost returned to its original value. No explanation can be given for such a phenomenon except the large variation in EC values. In the case of the cardiac muscle slurry the effect of the enzyme is very limited as revealed by the viscosity of the slurry; similarly, there is variation in EC but no trend. Even under the very controlled conditions prevailing in this last method, no improvement was obtained in the results as will be shown in the next section.

TABLE 47. Effect of enzymatic digestion on viscosity and EC of a skeletal muscle slurry by Method III

Time	Viscosity	Oil/slurry	EC
hours	relative units	g/g	g oil/mg protein
0	2,56	9,53	5,66
3	2,52	11,10	6,08
5	2,36	10,72	5,84
7	2,28	9,67	5,10
9	2,16	10,00	5,31
11	1,92	9,11	4,78
24	1,16	8,13	4,16
31	1,12	11,38	5,15
48	1,12	10,72	5,40

0,02% enzyme on a slurry basis (0,02 g protein/g slurry)
pH 6.0, 2°C, under agitation

TABLE 48. Effect of enzymatic digestion on viscosity and EC of a cardiac muscle slurry by Method III

Time	Viscosity	Oil/slurry	EC
hours	relative units	g/g	g oil/mg protein
0	1,06	10,64	5,40
3	1,32	10,83	5,39
5	1,22	11,02	5,52
7	1,14	10,36	5,21
9	1,08	10,11	4,99
11	1,08	10,77	5,19
23	0,98	10,21	5,62
36	0,98	10,72	5,10

0,02% enzyme on a slurry basis (0,02 g protein/g slurry)
pH 6.0, 2°C, under agitation

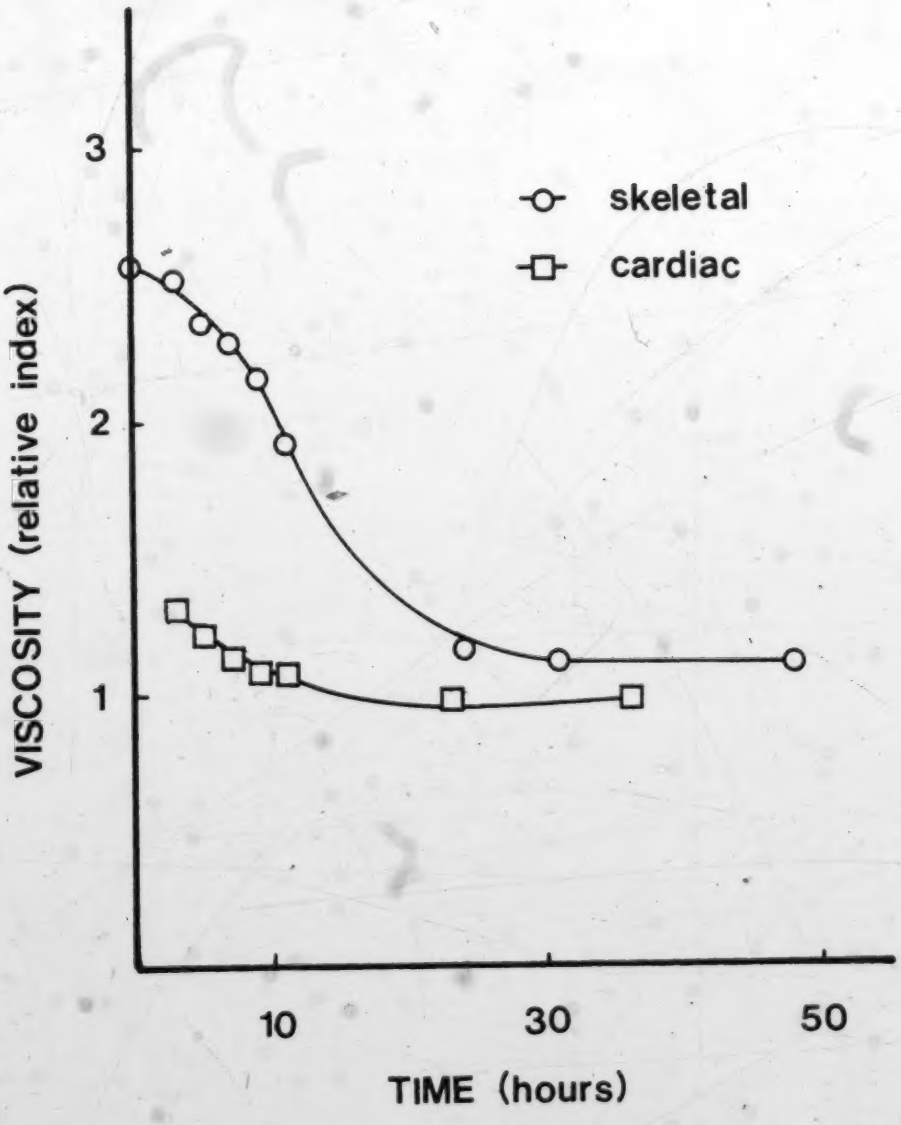

Figure 41: Effect of proteolysis on the viscosity of skeletal and cardiac muscle slurries

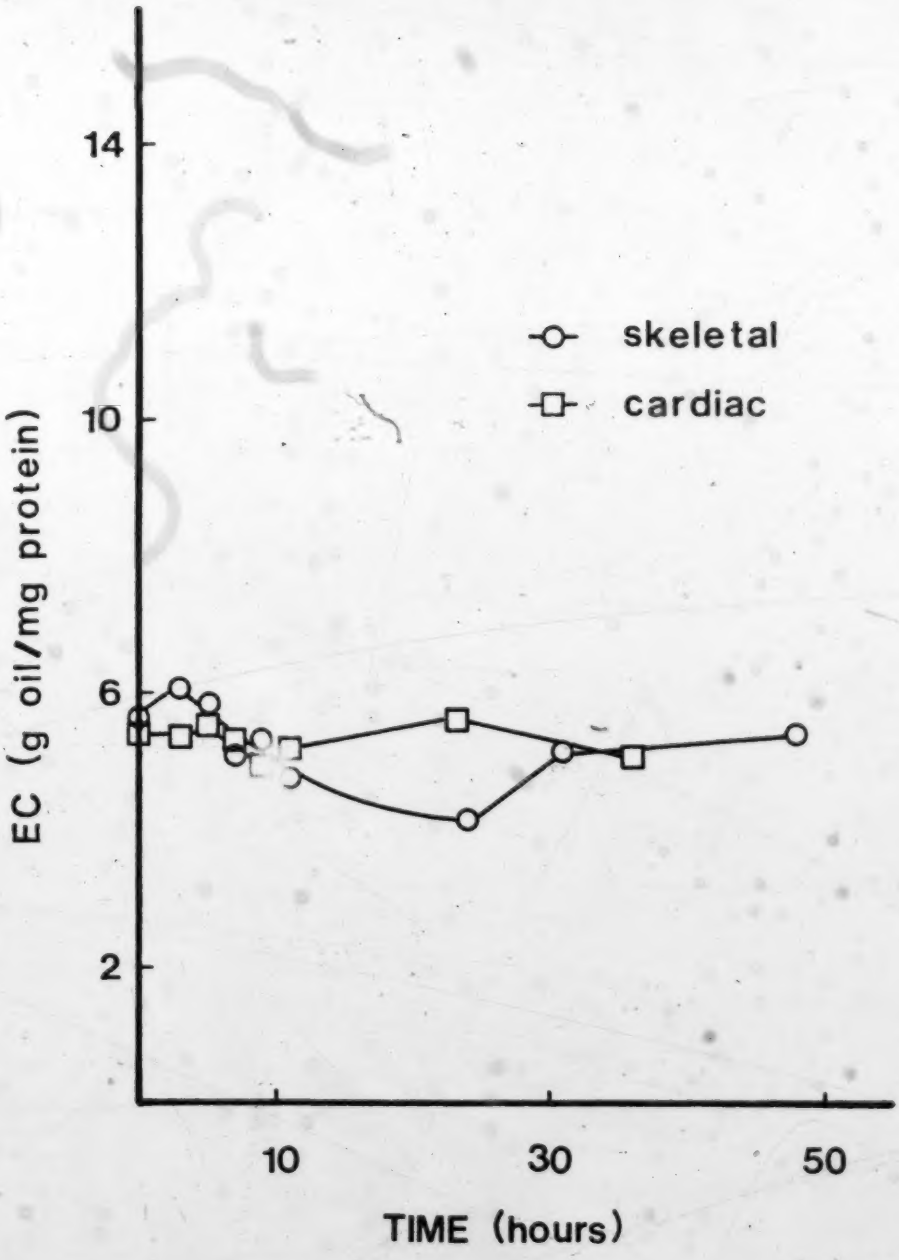

Figure 42: Effect of proteolysis on the EC of skeletal and cardiac muscle slurries by Method III

Evaluation of Method III

Under standard conditions of rate of oil addition, initial volume of solution, initial volume of oil, salt concentration, pH and speed of mixing, the EC of various solutions from skeletal and cardiac muscles was measured. First, the EC of skeletal and cardiac muscle slurries was determined; a small difference was found between the two muscles, the EC of the cardiac muscles slurries being slightly higher than that of the skeletal muscle slurries (tables 39, 40, 41, 42, figures 31, 33, 35). The difference between skeletal and cardiac muscles was not obvious at first because comparison was made of EC values at different protein levels since EC varies with protein concentration (figures 31, 33). However, when comparing values obtained at the same protein level, the EC of cardiac muscle slurries was slightly higher than that of skeletal muscle slurries (figure 35). On the other hand, no change in EC due to preblending was observed for both muscles (tables 39, 40) and no evident relationship was found between EC and enzymatic hydrolysis as followed by the viscosity of the slurries (tables 47, 48, figures 41, 42). Then, when the EC of meat protein fractions was determined, the water-soluble proteins were found to be more efficient than the salt-soluble proteins (tables 43, 44, 45, 46). Furthermore, the EC of protein fractions from the cardiac muscle was slightly higher than that of protein fractions from the skeletal muscle (figures 37, 39).

These results are totally different from the ones reported for other model systems by other workers and, more important, these results are different from practical knowledge. Using different model systems, several researchers have reported the cardiac muscle to have a lower

EC than the skeletal muscle (table 11). Based on practical experience, any sausage maker would agree that heart muscle is a poor emulsifier compared to lean meat; therefore, the amount of heart muscle in a formulation is always kept low to avoid binding problems. Similarly, I observed a difference in the viscosity of the slurries prepared from skeletal and cardiac muscles; whereas, the viscosity of cardiac muscle slurries is low, skeletal muscle slurries exhibit a high viscosity, being gel-like in appearance. Based on the experience acquired in this work on model systems, I believe that the discrepancy with actual sausage production arises from the large number of parameters involved and from the nature of the emulsions produced.

SUMMARY AND CONCLUSIONS

Problems were encountered with each one of the methods used in the present study. Results obtained can be summarized as follows.

Using the Waring Blendor technique, no large variation in the EC values and in the amounts of oil emulsified was observed except in two cases which are presented in table 49. However, for most experiments carried out using the Waring Blendor method, it did not seem to differentiate between the samples. This is illustrated by the results of numerous experiments a list of which is presented in table 50.

TABLE 49. Factors producing a large variation in EC as measured by Method I

1. Protein concentration
- EC decreases as the protein concentration is increased
- more oil is added as the protein concentration is increased
2. Muscle protein fractions
- myofibrillar proteins are more efficient than sarcoplasmic proteins

TABLE 50. Factors producing little or no variation in EC as measured by Method I

1.	Preliminary experiments
	- no difference in EC between skeletal and cardiac muscles
	- no increase in EC for skeletal muscle after preblending
2.	Various protein sources
	- emulsified similar amounts of oil
3.	Enzymatic digestion
	- no trend in EC and amount of oil emulsified
4.	Comparison of two muscles
	- small difference in EC, cardiac muscle being slightly superior to skeletal muscle
	- protein fractions from cardiac muscle are more efficient than those from skeletal muscle
5.	Preblending
	- only a small increase in EC after preblending
6.	pH
	- no effect on EC of skeletal muscle
	- steady increase with pH for cardiac muscle

Using the Lightnin stirrer, the effect of various factors on EC was determined. The results of these experiments are summarized in table 51. Lack of control over the speed of mixing lead to the use of a different mixer in Method III.

TABLE 51. Effect of various factors on EC as measured by Method II

1. Salt solutions
- large amount of oil for salt alone
- no effect of concentration
- no effect of pH
- no effect of rate of oil addition
2. Buffer solutions
- large amount of oil for buffer alone
- less oil as the concentration is decreased
3. Speed of mixing
- less oil at high speed of mixing
4. Initial volume of solution
- EC increases with initial volume of solution
5. Protein concentration (myofibrils)
- more oil as the protein concentration is increased
- EC decreases as the protein concentration is increased

And again with Method III, the effect of various factors on EC was investigated this time using a Stedi Speed stirrer and a standard protein BSA (table 52).

As it has been mentioned earlier, these results comparing skeletal and cardiac muscles are in disagreement with those reported for other model systems and with practical knowledge. This discrepancy was attributed to the large number of parameters involved and to differences in the nature of the emulsions produced under each set of conditions.

TABLE 52. Effect of various factors on EC as measured by Method III

1. Rate of oil addition	
- more oil as the rate of oil addition is increased	
2. Initial volume	
- solution: more oil as the initial volume is increased	
- oil: no effect on the amount of oil	
3. Salt	
- without protein: no effect of salt concentration	
- with protein (BSA): less oil as salt concentration is increased	
4. pH	
- without protein: large variation in the results	
- with protein (BSA): amount of oil minimal at pH 7.5	
5. Tripolyphosphate	
- slight increase in the amount of oil as the concentration is increased	
6. Protein concentration	
- BSA	EC decreases and oil increases with protein concentration
- skeletal and cardiac muscles slurries	
- sarcoplasmic and myofibrillar proteins	
7. Comparison of two muscles	
- EC of cardiac muscle slurries is slightly superior to that of skeletal muscle slurries	
- protein fractions from cardiac muscle are more efficient than those from skeletal muscle	
8. Preblending	
- no effect on EC	
9. Enzymatic digestion	
- no trend in EC	

There is no need to demonstrate the effect of the numerous parameters affecting the value of EC, this having been done throughout the present study. The fact that there are a large number of factors affecting EC is further illustrated by a comparison of the results obtained using two different methods previously described in this work. In figures 43 and 44, EC values of sarcoplasmic and myofibrillar proteins from both skeletal and cardiac muscles are compared. The values of EC obtained using the propeller technique are higher in the case of the sarcoplasmic proteins (figure 43). The difference is not as marked for the myofibrillar proteins, comparable EC values being obtained for the two different techniques (figure 44). This difference in the response obtained by two similar methods shows the difficulty of comparing results from different studies conducted under slightly different conditions. For this reason, every system must be defined very precisely. The preceding comparison emphasizes the importance of developing a standard method for the determination of EC.

The nature of the emulsions formed by the various protein slurries and protein fractions is different but the titration of these solutions with oil is always called EC even if different phenomena might be involved. For example, relatively large amounts of oil can be added to a salt solution but the titration of the so-called emulsion cannot be interrupted since the emulsion collapses right away without any further addition of oil. In the presence of protein, the titration can be interrupted without causing the breakdown of the emulsion. These two phenomena are obviously different as further evidenced by a different viscosity of the solutions but they can be measured the

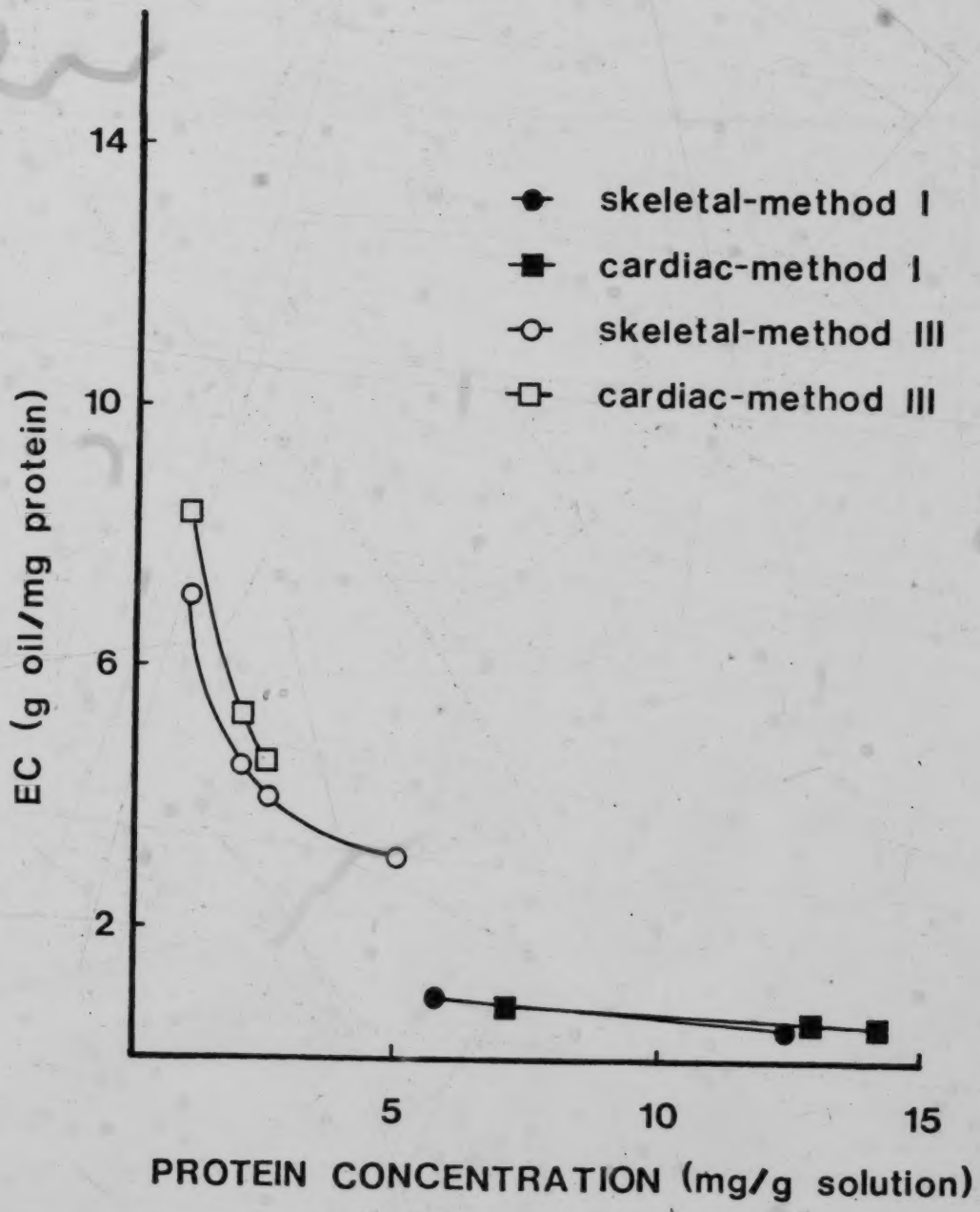

Figure 43: Comparison between the EC of sarcoplasmic proteins from skeletal and cardiac muscles as determined by the Waring Blendor (Method I) and the propeller (Method III) techniques

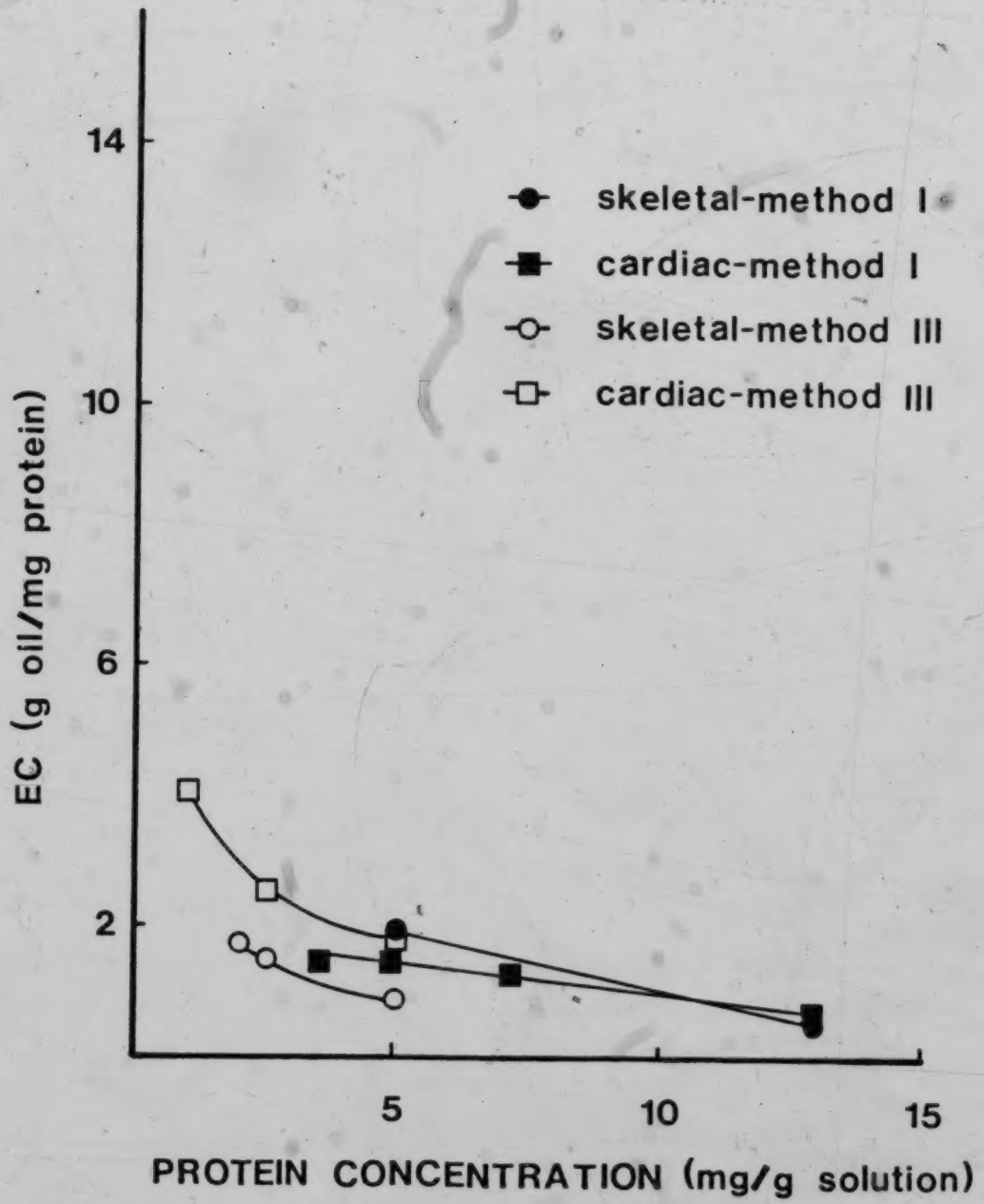

Figure 44: Comparison between the EC of myofibrillar proteins from skeletal and cardiac muscles as determined by the Waring Blendor (Method I) and the propeller (Method III) techniques

same way because both starting solutions exhibit low resistance to the passage of the current while after addition of oil, much higher resistance is noted.

In the presence of protein, the nature of the emulsion is influenced by numerous factors such as speed of mixing and protein concentration (tables 2, 4, from Swift *et al.* 1961 and personal observations). This point will not be elaborated here since it has already been discussed throughout this thesis. It suffices to give an example concerning the nature of the emulsions produced at different speeds of mixing and different protein concentrations. At low speed of mixing, the fat globules were large and as the speed was increased, the grain of the emulsion became finer, the size of the fat globules being reduced; in the latter case, the emulsion was very smooth and gel-like in appearance. As it was previously mentioned, the type of mixing was also found to have an effect on the nature of the emulsions since the viscosity of the emulsions produced using a Waring Blendor was low while the emulsions produced using a propeller were thicker even at lower protein concentrations. The effect of protein concentration on the nature of the emulsion became obvious when the standard protein BSA was first used. The first trials were to determine which protein range would require reasonable amounts of oil, starting with comparable protein concentrations to the ones used for meat proteins. At this protein level, emulsions resistant to flowing were formed; the viscosity of the emulsions was considerably reduced at lower protein levels and hence at shorter times of mixing. Similar observations were reported by Galluzzo and Regenstein (1978a) with respect to composition of the emulsion and time of mixing (table 53, from Galluzzo and Regenstein 1978a).

TABLE 53. Characteristics of myosin emulsions prepared with various oil-water ratios.

Sample volume ml	Oil volume ml	Mixing time min	Observations
5	5	1	Large foam beads (ca 30%)
5	8	1	Fine emulsion, few air bubbles
4	6	1	Some foam and separation
4	8	1	Good emulsion, but incomplete mixing
3	5	1	Good emulsion, slight separation, few air bubbles
3	5	5	Very fine and thick
3	6	1	Fine emulsion, few air bubbles on separation
3	6	5	Very fine, pours slowly
3	8	1	Fine emulsion but incomplete mixing
3	8	5	Fine emulsion, does not pour
2	5	5	Very fine, does not pour

(From Galluzzo and Regenstein 1978a)

Further evidence of the different nature of the emulsions was provided during the comparative study of the EC of sarcoplasmic and myofibrillar proteins under constant speed conditions (Method III). When the EC of solutions containing myofibrillar proteins was measured, the resistance of the initial solution containing the buffer and the myofibrils was low and remained low as the oil was added; at emulsion

collapse, the resistance increased sharply to reach infinity. On the other hand, with sarcoplasmic proteins, the low resistance of the initial solution containing buffer and proteins increased gradually as the oil was added to reach a very high electric resistance value; the situation was rather confusing as to whether or not this represented the end-point since as more oil was added, the resistance approached infinity.

It is therefore believed that the nature of the emulsions produced during EC determinations is quite different in each case. It appears that different proteins produce emulsions of different character, over a broad spectrum of physical properties and the oil titration end-point of these different emulsions is also different. Therefore, comparison of EC values is devoid of significance since there is no constancy in the conditions of determination and consequently in the results obtained. However, the effect of protein concentration on EC was constant, the EC decreasing as the protein concentration was increased. This effect of protein concentration on EC is due to the definition of EC itself, EC being expressed as the amount of oil per unit of protein, and as such has no intrinsic meaning or significance.

The present work demonstrates that the conventional measurement of EC of proteins is meaningless because it is difficult to control all the variables and compare the results. As well, this work emphasizes the need for a new method to evaluate the emulsifying properties of proteins in emulsion products.

BIBLIOGRAPHY

ACTON, J.C., SAFFLE, R.L. 1972. Emulsifying capacity of muscle protein: Phase volumes at emulsion collapse. J. Food Sci. 37: 904.

BALIGA, B.R., MADAIAH, N. 1970. Quality of sausage emulsion prepared from mutton. J. Food Sci. 35: 383.

BECHER, P. 1965. "Emulsions: Theory and Practice." 2nd Ed. p. 2. Reinhold Publishing Corporation, New York.

BORCHERT, L.L., GREASER, M.L., BARD, J.C., CASSENS, R.G., BRISKEY, E.J. 1967. Electron microscopy of a meat emulsion. J. Food Sci. 32: 419.

BORTON, R.J., WEBB, N.B., BRATZLER, L.J. 1968. Emulsifying capacities and emulsion stability of dilute meat slurries from various meat trimmings. Food Technol. 22: 506.

BRISKEY, E.J. 1970. Functional evaluation of protein in food systems. In: Evaluation of novel protein products. Bender, A. *et al.* Eds. Pergamon Press, London, 2.

BROWN, D.D., TOLEDO, R.T. 1975. Relationship between chopping temperatures and fat and water binding in comminuted meat batters. J. Food Sci. 40: 1061.

CARPENTER, J.A., SAFFLE, R.L. 1964. A simple method of estimating the emulsifying capacity of various sausage meats. J. Food Sci. 29: 774.

CARPENTER, J.A., SAFFLE, R.L. 1965. Some physical and chemical factors affecting the emulsifying capacity of meat protein extracts. Food Technol. 19: 1567.

CHRISTIAN, J.A., SAFFLE, R.L. 1967. Plant and animal fats and oils emulsified in a model system with muscle salt-soluble protein. Food Technol. 21: 1024.

CRENWELGE, D.D., DILL, C.W., TYBOR, P.T., LANDMANN, W.A. 1974. A comparison of the emulsification capacities of some protein concentrates. J. Food Sci. 39: 175.

CUNNINGHAM, F.E., FRONING, G.W. 1972. A review of factors affecting emulsifying characteristics of poultry meat. Poultry Sci. 51: 1714.

DONNELLY, T.H., RONGEY, E.H., BARSUKO, V.J. 1966. Protein composition and functional properties of meat. J. Agr. Food Chem. 14: 196.

DU BOIS, M.W., ANGLEMIER, A.F., MONTGOMERY, M.W., DAVIDSON, W.D. 1972. Effect of proteolysis on the emulsification characteristics of bovine skeletal muscle. J. Food Sci. 37: 27.

FRONING, G.W., ANDERSEN, J., MEBUS, C.A. 1970. Histological characteristics of turkey meat emulsions. Poultry Sci. 49: 497.

FRONING, G.W., JOHNSON, F. 1973. Improving the quality of mechanically deboned fowl meat by centrifugation. J. Food Sci. 38: 279.

FRONING, G.W., NEELAKANTAN, S. 1971. Emulsifying characteristics of prerigor and postrigor poultry muscle. Poultry Sci. 50: 839.

FRONING, G.W., SATTERLEE, L.D., JOHNSON, F. 1973. Effect of skin content prior to deboning on emulsifying and color characteristics of mechanically deboned chicken back meat. Poultry Sci. 52: 923.

FUKAZAWA, T., HASHIMOTO, Y., YASUI, T. 1961. Effect of some proteins on the binding quality of an experimental sausage. J. Food Sci. 26: 541.

GALLUZZO, S.J., REGENSTEIN, J.M. 1978a. Emulsion capacity and timed emulsification of chicken breast muscle myosin. J. Food Sci. 43: 1757.

GALLUZZO, S.J., REGENSTEIN, J.M. 1978b. Role of chicken breast muscle proteins in meat emulsion formation: myosin, actin and synthetic actomyosin. J. Food Sci. 43: 1761.

GALLUZZO, S.J., REGENSTEIN, J.M. 1978c. Role of chicken breast muscle proteins in meat emulsion formation: natural actomyosin, contracted and uncontracted myofibrils. J. Food Sci. 43: 1766.

GILLETT, T.A., MEIBURG, D.E., BROWN, C.L., SIMON, S. 1977. Parameters affecting meat protein extraction and interpretation of model system data for meat emulsion formation. J. Food Sci. 42: 1606.

GOLL, D.E., ROBSON, R.M. 1967. Molecular properties of post-mortem muscle. I. Myofibrillar nucleosidetriphosphatase activity of bovine muscle. J. Food Sci. 32: 323.

GRABOWSKA, J., SIKORSKI, Z. 1974. The emulsifying capacity of fish proteins. Proc. IV Int. Congr. Food Sci. Technol., Vol. 2: 13.

HANSEN, L.J. 1960. Emulsion formation in finely comminuted sausage. Food Technol. 14: 565.

HAQ, A., WEBB, N.B., WHITFIELD, J.K., HOWELL, A.J., BARBOUR, B.C. 1973. Measurement of sausage emulsion stability by electrical resistance. J. Food Sci. 38: 1224.

HAY, J.D., CURRIE, R.W., WOLFE, F.H. 1973. Effect of postmortem aging on chicken muscle fibrils. J. Food Sci. 38: 981.

HEGARTY, G.R., BRATZLER, L.J., PEARSON, A.M. 1963. Studies on the emulsifying properties of some intracellular beef muscle proteins. J. Food Sci. 28: 663.

HELMER, R.L., SAFFLE, R.L. 1963. Effect of chopping temperature on the stability of sausage emulsions. Food Technol. 17: 1195.

HUDSPETH, J.P., MAY, K.N. 1967. A study of the emulsifying capacity of salt soluble proteins of poultry meat. I. Light and dark meat tissues of turkeys, hens and broilers, and dark meat tissues of ducks. Food Technol. 21: 1141.

HUDSPETH, J.P., MAY, K.N. 1969. Emulsifying capacity of salt-soluble proteins of poultry meat. II. Heart, gizzard and skin from broilers, turkeys, hens and ducks. Food Technol. 23: 373.

HUFFMAN, V.L., LEE, C.K., BURNS, E.E. 1975. Selected functional properties of sunflower meal (*Helianthus annuus*). J. Food Sci. 40: 70.

INKLAAR, P.A., FORTUIN, J. 1969. Determining the emulsifying and emulsion stabilizing capacity of protein meat additives. Food Technol. 23: 103.

IVEY, F.J., WEBB, N.B., JONES, V.A. 1970. The effect of disperse phase droplet size and interfacial film thickness on the emulsifying capacity and stability of meat emulsions. Food Technol. 24: 1279.

JOHNSON, R.G., HENRICKSON, R.L. 1970. Effect of treatment of pre- and postrigor porcine muscles with low sodium chloride concentrations on the subsequent extractability of proteins. J. Food Sci. 35: 268.

KINSELLA, J.E. 1976. Functional properties of proteins in foods: A survey. Crit. Rev. Food Sci. Nutr. 7: 219.

KOURY, B.J., SPINELLI, J. 1975. Effect of moisture, carbohydrate and atmosphere on the functional stability of fish protein isolates. J. Food Sci. 40: 58.

LIN, M.J.Y., HUMBERT, E.S., SOSULSKI, F.W. 1975. Quality of wieners supplemented with sunflower and soy products. Can. Inst. Food Sci. Technol. J. 8: 97.

MARSHALL, W.H., DUTSON, T.R., CARPENTER, Z.L., SMITH, G.C. 1975. A simple method for emulsion end-point determinations. J. Food Sci. 40: 896.

MATTIL, K.F. 1971. The functional requirements of proteins for foods. Am. Oil Chem. Soc. J. 48: 477.

MAURER, A.J., BAKER, R.C. 1966. The relationship between collagen content and emulsifying capacity of poultry meat. Poultry Sci. 45: 1317.

MAURER, A.J., BAKER, R.C., VADEHRA, D.V. 1969a. Kind and concentration of soluble protein extract and their effect on the emulsifying capacity of poultry meat. Food Technol. 23: 575.

MAURER, A.J., BAKER, R.C., VADEHRA, D.V. 1969b. The influence of type of poultry and carcass part on the extractability and emulsifying capacity of salt-soluble proteins. Poultry Sci. 48: 994.

McCREADY, S.T., CUNNINGHAM, F.E. 1971a. Properties of salt-extractable proteins of broiler meat. J. Sci. Food Agric. 22: 317.

McCREADY, S.T., CUNNINGHAM, F.E. 1971b. Salt-soluble protein of poultry meat. I. Composition and emulsifying capacity. Poultry Sci. 50: 243.

MEYER, J.A., BROWN, W.L., GILTNER, N.E., GUINN, J.R. 1964. Effect of emulsifiers on the stability of sausage emulsions. Food Technol. 18: 1796.

MORRISON, G.S., WEBB, N.B., BLUMER, T.N., IVEY, F.J., HAQ, A. 1971. Relationship between composition and stability of sausage-type emulsions. J. Food Sci. 36: 426.

NEELAKANTAN, S., FRONING, G.W. 1971. Studies on the emulsifying characteristics of some intracellular turkey muscle proteins. J. Food Sci. 36: 613.

NEER, K.L., PLIMPTON, R.F. Jr., OCKERMAN, H.W. 1974. Bologna product characteristics as influenced by various sources and levels of cottage cheese whey. J. Food Sci. 39: 993.

NOBLE, J. 1973. Machine deboning turns poultry by-product into sausage ingredient. Food Product Develop. 7 (8): 102.

PARKES, M.R., MAY, K.N. 1968. Effect of freezing, evaporation and freeze-drying on emulsifying capacity of salt-soluble protein. Poultry Sci. 47: 1236.

PEARSON, A.M., SPOONER, M.E., HEGARTY, G.R., BRATZLER, L.J. 1965. The emulsifying capacity and stability of soy sodium proteinate, potassium caseinate and nonfat dry milk. Food Technol. 19: 1841.

PUSKI, G. 1976. A review of methodology for emulsification properties of plant proteins: Cereal Chem. 53: 650.

PUSKI, G. 1975. Modification of functional properties of soy proteins by proteolytic enzyme treatment. Cereal Chem. 52: 655.

QUINN, M.R., BEUCHAT, L.R. 1975. Functional property changes resulting from fungal fermentation of peanut flour. J. Food Sci. 40: 475.

RAMANATHAM, G., RAN, L.H., URS, L.N. 1978. Emulsification properties of groundnut protein. J. Food Sci. 43: 1270.

RANDALL, C.J. 1977. Use of mechanically deboned and manually deboned poultry meat in meat emulsions: A review. Can. Inst. Food Sci. Technol. J. 10: 147.

RANDALL, C.J. 1978. Sausage technology. Part I. The chemistry of meat emulsions. QC - R & D 5(1): 2.

SAFFLE, R.L. 1968. Meat emulsions. Adv. Food Res. 22: 105.

SAFFLE, R.L., CHRISTIAN, J.A., CARPENTER, J.A., ZIRKLE, S.B. 1967. Rapid method to determine stability of sausage emulsions and effects of processing temperatures and humidities. Food Technol. 21: 784.

SAFFLE, R.L., GALBREATH, J.W. 1964. Quantitative determination of salt-soluble protein in various types of meat. Food Technol. 18: 1943.

SATTERLEE, L.D., FREE, B., LEVIN, E. 1973. Utilization of high protein tissue powders as a binder/extender in meat emulsions. J. Food Sci. 38: 306.

SATTERLEE, L.D., FRONING, G.W., JANKY, D.M. 1971. Influence of skin content on the composition of mechanically deboned poultry meat. J. Food Sci. 36: 979.

SCHUT, J. 1978. Basic meat emulsion technology. In: "Proc. Meat Industry Res. Conference", p. 1. American Meat Institute Foundation, Arlington, Virginia.

SCHUT, J. 1976. Meat emulsions. In: "Food Emulsions". Ed. Friberg, S. p. 385. Marcel Dekker Inc., U.S.A.

SMITH, G.C., JUHN, H., CARPENTER, Z.L., MATTIL, K.F., CATER, C.M. 1973. Efficacy of protein additives as emulsion stabilizers in frankfurters. J. Food Sci. 38: 849.

SPINELLI, J., KOURY, B., MILLER, R. 1972a. Approaches to the utilization of fish for the preparation of protein isolates. Enzymic modifications for myofibrillar fish proteins. J. Food Sci. 37: 604.

SPINELLI, J., KOURY, B., MILLER, R. 1972b. Approaches to the utilization of fish for the preparation of protein isolates. Isolation and properties of myofibrillar and sarcoplasmic fish proteins. J. Food Sci. 37: 599.

SULZBACHER, W.L. 1973. Meat emulsions. J. Sci. Food Agric. 24: 589.

SWIFT, C.E. 1965. The emulsifying properties of meat protein. In: "Meat emulsions". Saffle, R.L. 1968. Adv. Food Res. 22: 105.

SWIFT, C.E., LOCKETT, C., FRYAR, A.J. 1961. Comminuted meat emulsions. The capacity of meats for emulsifying fat. Food Technol. 15: 468.

SWIFT, C.E., SULZBACHER, W.L. 1963. Comminuted meat emulsions: Factors affecting meat proteins as emulsion stabilizers. Food Technol. 17: 224.

TORNBERG, E., HERMANSSON, A.M. 1977. Functional characterization of protein stabilized emulsions: Effect of processing. J. Food Sci. 42: 468.

TORTEN, J., WHITAKER, J.R. 1964. Evaluation of the biuret and dye-binding methods of protein determination in meats. J. Food Sci. 29: 168.

TOWNSEND, W.E., WITNAUER, L.P., RILOFF, J.A., SWIFT, C.E. 1968. Comminuted meat emulsions: Differential thermal analysis of fat transitions. Food Technol. 22: 319.

TRAUTMAN, J.C. 1964. Fat-emulsifying properties of prerigor and postrigor pork proteins. Food Technol. 18: 1065.

TSAI, R., CASSENS, R.G., BRISKEY, E.J. 1972. The emulsifying properties of purified muscle proteins. J. Food Sci. 37: 286.

TURNER, E.W., OSLON, F.C. 1959. Manufacture of sausage and ground meat products. US Patent No. 2,874,060. In: "Protein composition and functional properties of meat", Donnelly, T.H. *et al.* 1966. J. Agr. Food Chem. 14: 196.

TYBOR, P.T., DILL, C.W., LANDMANN, W.A. 1973. Effect of decolorization and lactose incorporation on the emulsifying capacity of spray-dried blood protein concentrate. J. Food Sci. 38: 4.

Van EERD, J.P. 1972. Emulsion stability and protein extractability of ovine muscle as a function of time postmortem. J. Food Sci. 37: 473.

WANG, J., KINSELLA, J.E. 1976. Functional properties of novel proteins: Alfafa leaf protein. J. Food Sci. 41: 286.

WEBB, N.B., IVEY, F.J., CRAIG, H.B., JONES, V.A., MONROE, R.J. 1970. The measurement of emulsifying capacity by electrical resistance. J. Food Sci. 35: 501.

YASUMATSU, K., SAWADA, K., MORITAKA, S., MISAKI, M., TODA, J., WADA, T., ISHII, K. 1972. Whipping and emulsifying properties of soybean products. Agric. Biol. Chem. 36: 719.

YOUNG, L.L. 1976. Composition and properties of an animal protein isolate prepared from bone residue. J. Food Sci. 41: 606.